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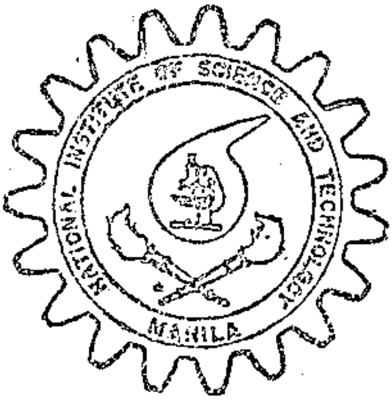
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THE PHILIPPINE JOURNAL OF SCIENCE

VOL. 102 MARCH-JUNE, 1973 NOS. 1-2

PHYTOCHEMICAL INVESTIGATION OF COLEUS BLUMEI BENTH.

I. PRELIMINARY STUDIES OF THE LEAVES

By LOURDES L. GARCIA, LUZ LL. COSME, HONORATA R. PERALTA, and
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National Institute of Science and Technology, Manila

SIX TEXT FIGURES

ABSTRACT

A long chain hydrocarbon, a sterol and triterpenoid compounds all unidentified, are reported for the first time from the leaves of *Coleus blumei* Benth. Flavonoid compounds were also detected. The ehtyl acetate soluble fraction of the ethanol extract showed activities against several microorganisms and Ehrlich ascites tumor cells.

This herb belonging to the mint family (Labiatæ) and popularly called *mayana* (Tagalog) in the Philippines is cultivated for ornamental purposes due to its wide variation in leaf color and also as a source of material for curing some common ailments. According to Guerrero (1921) its pounded leaves are valuable as a cure for headaches and for healing bruises, while Burkill and Haniff (1930) recorded its use in the form of decoction for dyspepsia and also as eye drop for ophthalmia. Even up to the present time most of the old folks from the Bicol, Tagalog, and Visayan regions make use of the leaves of this plant as a very common remedy for bruises, boils, and wounds.

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Tolbert *et al* (1957) reported the presence of sedoheptulose, a seven-carbon sugar in the leaves of this plant. Two new leaf pigments Coleon A and Coleon B were also isolated from this plant by Eugester *et al* (1963). Several flavonoid pigments were identified by Palmierie and Landi (1965) in the leaves of two *C. blumei* cultivars of Italy. It was observed that there was very little information concerning the chemical constituents of this plant. Furthermore, investigations associating these chemical constituents to the therapeutic value of *Coleus blumei* Benth. as a folkloric remedy have not been undertaken.

This paper presents a method of extraction, fractionation and isolation of the components of the leaves of the purple variety of *mayana*. Preliminary testing of the crude fractions for their antibacterial and antitumor activities against 12 microorganisms and Ehrlich ascites tumor cells respectively was done.

MATERIALS AND METHODS

The leaves of the purple variety of *mayana* plants grown in Mandaluyong, Rizal were used in the preparation of the extracts for analysis. The freshly collected leaves (5 kg) air dried for 5 days yielded a partially dried material (2 kg), containing 35-per cent moisture. All the solvents used were of reagent grade except for the ethyl acetate which had to be made neutral and distilled before use. This was done by mixing 200 ml of ethyl acetate (A.R.) with 30 ml of 5-per cent aqueous solution of Na_2CO_3 in a separatory funnel. The ethyl acetate layer was separated, washed with distilled water until neutral and then vacuum distilled on a water bath. The petroleum ether with a boiling point of 30° to 60°C was used. Silica gel G plates prepared had a thickness of about 0.25 mm, using silica gel G according to Stahl (Merck).

Chromatographic isolation of compounds and the determination of their **IR** spectra in Nujol were undertaken by the senior author at the Tropical Products Institute, London, England.

Preparation of extracts.—The partially air dried plant material (2 kg) was cut into small pieces, placed in Erlenmeyer flasks (3-liter capacity) and extracted with 95-per cent ethanol by refluxing on the steam bath for 12 hrs. The solvent was changed every 4 hours to prevent excessive heating of the extract. All of the ethanol extracts were combined, filtered and concentrated by vacuum distillation at 65°C. The concentrate was further dried in a vacuum oven at the same temperature yielding 80 g of a dark colored thick gummy residue. This residue was transferred into a flask and refluxed with freshly distilled neutral ethyl acetate for 12 hours on a steam bath, changing the solvent every 3 hours. The combined ethyl acetate extracts were dried *in vacuo* yielding 12 g of a brown semisolid residue, Fraction A. The material remaining after the ethyl acetate extraction was then collected, vacuum dried and labeled Fraction B. (Fig. 1.)

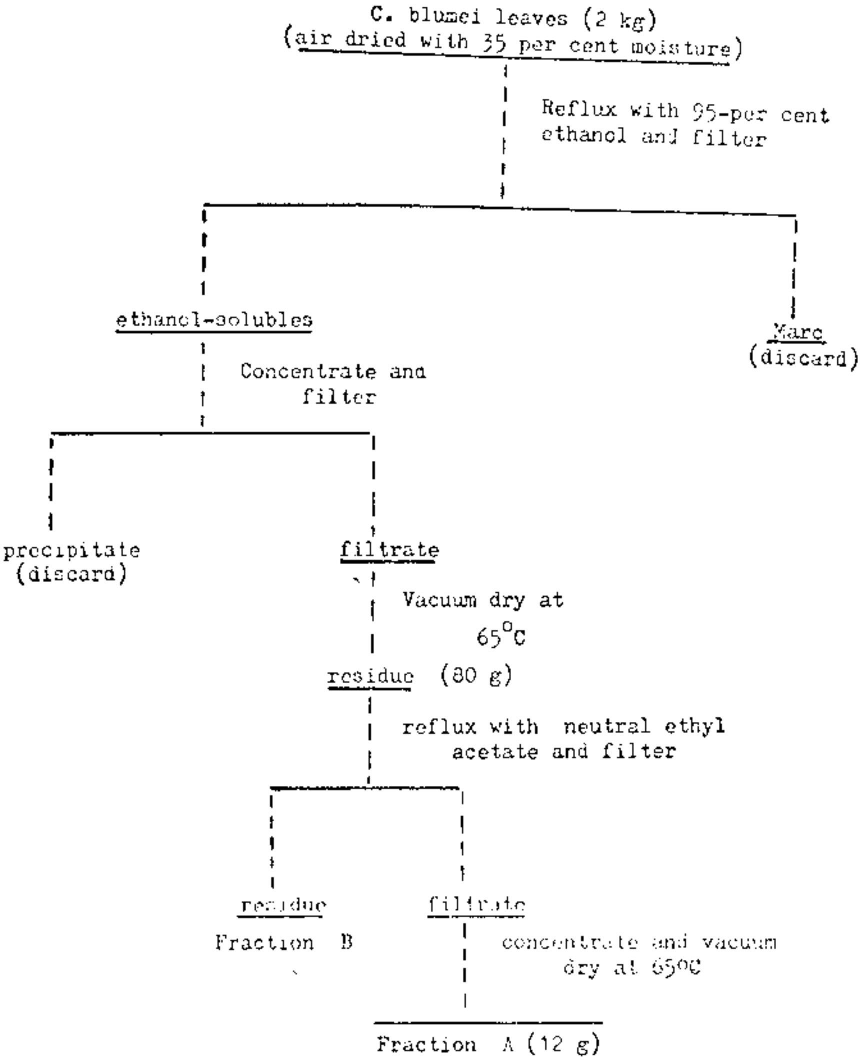

FIG. 1. Fractionation scheme for *Coleus blumei* leaves.

Chromatographic screening of crude extracts.—Thin-layer chromatograms of the crude extracts, Fractions A and B were prepared on silica gel G plates. Solvent mixtures, petroleum ether-ethyl ether (50:50) and chloroform-methanol (5:95) were used as developing solvents. The chromatoplates were sprayed with 50-per cent aqueous solution of H_2SO_4 and allowed to dry in the oven at 120°C for 20 minutes. The located spots were observed and illustrated in Fig. 2.

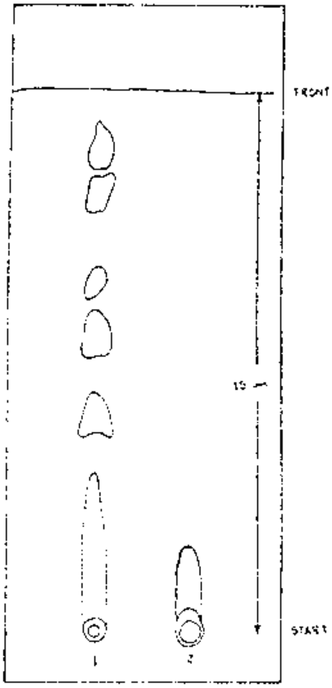

FIG. 2. Thin-layer chromatogram of crude extracts of *Coleus blumei* Benth. 1—Fraction A, 2—Fraction B adsorbent: silica gel. solvent: petroleum ether-ethyl ether (50:50) indicator: charring with 50-per cent sulfuric acid solution.

Column chromatography of Fraction A.—This ethyl acetate soluble fraction gave characteristic colors with Liebermann-Burchard reaction and Salkowski test suggestive of the presence of triterpenoid and/or steroidal compounds (Paech and Tracey, 1955). Resolution of this fraction on a column of aluminum oxide powder (Baker Analyzed Reagent, suitable for chromatographic use) did not produce good separation of the components. A description of the isolation of the components on a column of silica gel follows.

The whole amount of this fraction (12 g) was chromatographed on a glass tube (5 by 60 cm) containing 250 g of silica gel (Merck, 80 to 200 mesh). This was first dissolved in a minimum amount of acetone and to the solution an equal amount of the adsorbent was added, mixed thoroughly until a homogenous mixture was obtained. The mixture was freed of the solvent by heating on a water bath, allowed to cool then added to the top of the column of silica gel. Elution of the column was initiated with petroleum ether followed by petroleum ether containing increasing quantities of ethyl ether, from 5 to 50 per cent and finally anhydrous ethyl ether. One hundred-ml fractions were collected and each fraction subjected to thin-layer chromatography on silica gel G plates. Fractions showing identical chromatograms were combined and evaporated to dryness in a rotary evaporator. The percentage of ethyl ether was increased the moment the fraction collected contained a very slight amount of the components as indicated by thin-layer chromatography. Two different solvent systems, petroleum ether-ethyl ether mixture (50:50) and methanol-chloroform mixture (5:95) were used as developing solvents. The chromatoplates were sprayed with 50-per cent H_2SO_4 solution and dried in the oven for 20 minutes at 120°C.

Isolation of Compounds a, b, c, and d.—The combined fractions eluted by petroleum ether alone and petroleum ether containing 5-per cent ethyl ether, yielded a white waxy residue upon evaporation. After repeated crystallization in hot chloroform and methanol, a white amorphous solid, Compound a, m.p. 65° to 70°C was obtained.

The fractions eluted by petroleum ether containing 10 and 20-per cent ethyl ether were combined and upon concentration gave an oily residue, Compound b, which failed to crystallize in the usual organic solvents.

The residue of the combined fractions eluted by petroleum ether containing 25- to 35-per cent ethyl ether was repeatedly crystallized in hot methanol obtaining a slightly colored crystalline solid, Compound c, melting at 148° to 150°C.

Combined fractions eluted by petroleum ether containing 40- to 50-per cent ethyl ether and ethyl ether (100 per cent) yielded a greenish-black residue upon evaporation to dryness. This material was redissolved in a minimum amount of hot methanol heated to boiling for 5 minutes with activated carbon and filtered. The filtrate obtained was concentrated until a slight turbidity was observed. The concentrate was placed in the cold to crystallize and the slightly colored crystalline solid that formed was collected. Repeated crystallization of this material in hot methanol yielded a crystalline solid Compound d, that melted at 240° to 250°C.

Thin-layer chromatography of the isolated compounds was run in two solvent systems, petroleum ether-ethyl ether (50:50) and methanol-chloroform (5:95), sprayed with 50-per cent H_2SO_4 solution and dried at 120°C for 20 minutes in a drying oven. These compounds were also subjected to color reaction tests and their infrared spectra were determined.

Examination of Fraction B.—A preliminary separation of this fraction was undertaken by the application of solvent extraction method of Farnsworth *et al* (1968). The crude fraction (50 g) was dissolved in hot water (1 liter) and the aqueous solution obtained was filtered and concentrated to about 200 ml in a rotary evaporator. The concentrate was extracted successively with chloroform, ethyl ether, ethyl acetate, and ethyl acetate-methanol mixture (90:10). The extracts obtained were dried over anhydrous sodium sulfate then evaporated to dryness.

All the four crude extracts dissolved in ethanol (95 per cent) were treated with the following reagents for color reaction tests for flavonoids: (*1*) ferric chloride, (*2*) magnesium-hydrochloric acid; (*3*) sodium hydroxide, and (*4*) neutral lead acetate. They were also subjected to ascending paper chromatography using n-butanol: 27-per cent aqueous acetic acid solution (50:50) as developing solvent with 2-per cent aluminum chloride solution (ethanolic) as spray reagent. The chromatograms were dried and examined in ordinary light and under ultraviolet light before and after spraying. Crystallization of the components of these fractions has not yet been attempted.

Biological studies.—Five-per cent solutions of crude Fractions A and B in methanol, were tested for their anticancer activity on Ehrlich ascites tumor cells (EATC) by the filter paper disc method of Masiluñgan *et al* (1964). Likewise they were tested for their antibacterial activity by following the modified disc agar plate method of Beer and Sherwood (1954) using the following test organisms, namely: (1) *Micrococus aureus*, (2) *Micrococcus aureus* resistant to penicillin, (3) *Alcaligenes faecales*, (4) *Mycobacterium tuberculosis-607*, (5) *Pseudomonas aeruginosa*, (6) *Escherichia coli*, (7) *Salmonella gallinarium*, (8) *Bacillus subtulis*, (9) *Candida albicans*, (10) *Caratestanella paradoxa*, (11) *Fusarium moniliforme*, and (12) *Saccharomyces cerivisiae*.

RESULTS AND DISCUSSION

Thin-layer chromatography examination of the crude extracts (Fig. 2) showed the presence of several components in Fraction A as indicated by the five definite spots and a streak produced. Fraction B remained at the origin. It was observed that more defined spots were produced by using petroleum ether-ethyl ether (50:50) as developing solvent.

With silica gel as adsorbent, column chromatography of Fraction A gave a satisfactory separation of Compounds a, b, c, and d. It was evidenced by TLC as shown in Fig. 3, that Compounds a, c, and d each contain a single component, while Compound b was a mixture of Compound a and two other components. Petroleum ether-ethyl ether (50:50) used as a developer produced defined spots of the compound.

The white amorphous solid, Compound a; m.p. 65° to 70°C, gave no color reaction with Liebermann-Burchard and Salkowski tests indicating that this isolate was not steroidal and/or triterpenoid in nature (Paech and Tracey, 1955). Thin-layer chromatography on silica gel G plates with petroleum ether-ethyl ether mixture (50:50) as developing solvent showed a broad, tapering spot near the solvent front (Fig. 3). Infrared spectrum of this isolate in Nujol (Fig. 4) showed a doublet at 730 to 720 cm^{-1}, representing $(CH_2)n$ skeletal vibration, a characteristic adsorption of a long chain hydrocarbon in the solid state (Nakanishi, 1962). The weak band at 3500 to 3300 cm^{-1} (VOH) might be attributed to the presence of moisture or alcohol in the sample.

Compound c is a crystalline solid that melts at 148° to 150°C. With the Salkowski reaction, a red color was slowly produced in the chloroform layer when concentrated sulfuric acid was added to a solution of the compound in chloroform. In the Liebermann-Burchard test, when the chloroform solution of the compound was treated with acetic anhydride and concentrated sulfuric acid, a light red color that immediately changed to blue and finally green was produced in the chloroform layer. Both color reactions produced indicated that the compound was steroidal in nature (Finar, 1964). Thin-layer chromatography of the isolate indicated the presence of only one compound (Fig. 3). Its infrared spectrum in Nujol (Fig. 5) showed absorption bands at 3500 to 3300 cm^{-1} (VOH), 1680 to 1640 cm^{-1} (VC=C), 1060 cm^{-1} (VC-O), and 965 cm^{-1} (d CH, out of plane), all representing characteristic group frequencies of a sterol compound (Bellamy, 1957).

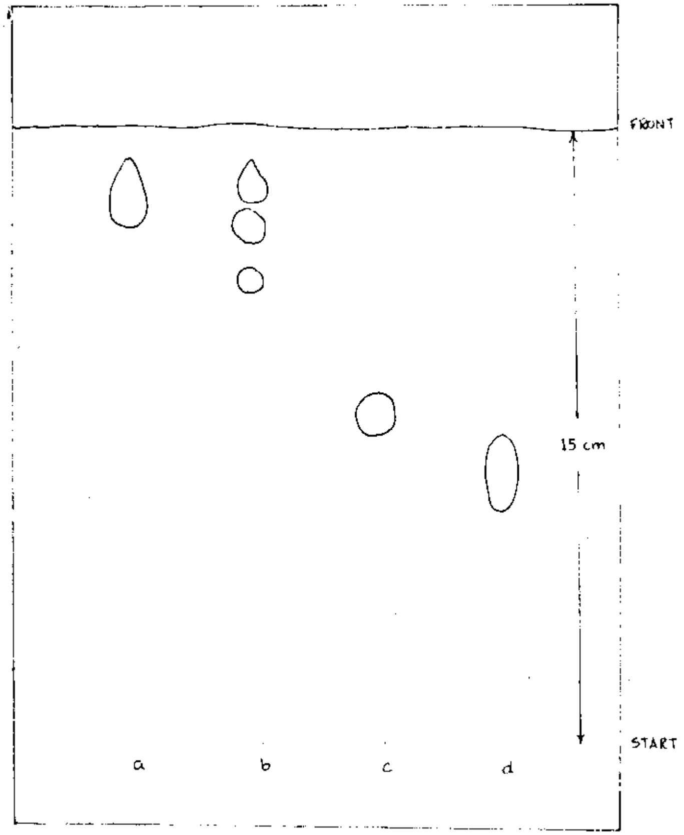

FIG. 3. Thin-layer chromatogram of compounds a, b, c, and d from the leaves of *Coleus blumei* Benth. adsorbent: silica gel G. solvent: petroleum ether-ethyl ether (50:50). Indicator: Charring with 50-per cent sulfuric acid solution.

Compound d, a white crystalline solid; m.p. 240° to 255°C showed a single elongated spot in TLC plate (Fig. 3). This isolate in chloroform solution gave a blue-green color with the Liebermann-Burchard test, and a purple color was produced when the test was applied directly to the compound on a white spotting

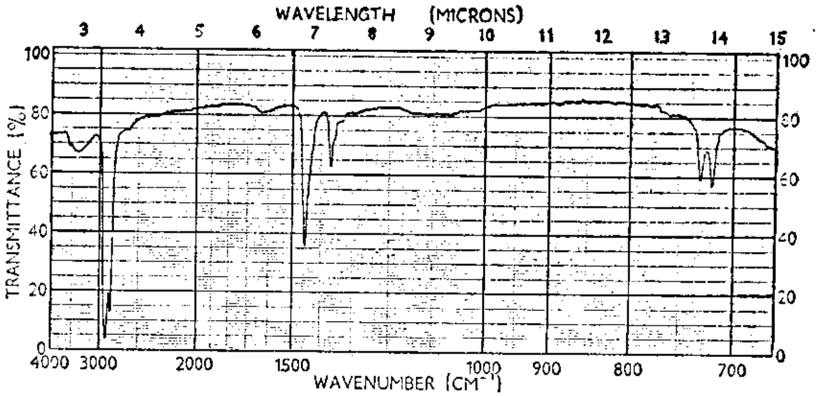

FIG. 4. IR Spectrum of compound a (Nujol).

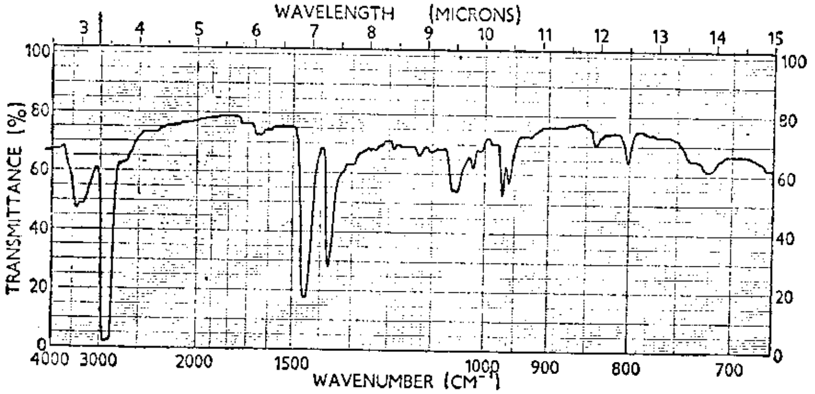

FIG. 5. IR Spectrum of compound c (Nujol).

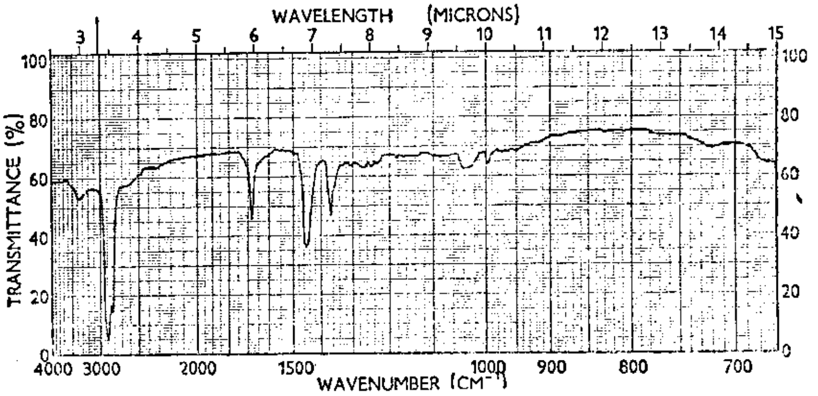

FIG. 6. IR Spectrum of compound d (Nujol).

plate. These characteristic color reactions produced indicated that the isolate was triterpenoid in nature (Sims *et al*, 1959). Infrared spectrum in Nujol (Fig. 6) showed peaks at 3400 to 3200 cm^{-1} (VOH) and at 1690 cm^{-1} (VC-O), but were not so significant. However, since its melting point had a very wide range and IR peaks were not so significant, it is possible that impurities are present or it might be a mixture of two or more components. Further purification has not been undertaken due to lack of sufficient isolated material.

The presence of flavonoid compounds in Fraction B was evidenced by color reaction tests and paper chromatography. Preliminary separation of this water-soluble fraction, yielded four extracts: (*a*) chloroform, (*b*) ethyl ether, (*c*) ethyl acetate, and (*d*) ethyl acetate-methanol mixture. The last three extracts produced the following colors: (*1*) green with ferric chloride solution, (*2*) orange-red with magnesium ribbon + concentrated hydrocloric acid, (*3*) yellow precipitate with sodium hydroxide solution, and (*4*) yellow with neutral lead acetate solution. These strongly indicated that these extracts contain flavonoid compounds particularly of the flavone type (Paech and Tracey, 1955). On the other hand, none of the tests produced any color reaction with the first extract. Results of paper chromatography showed two visible and fluorescent spots with Rf values, 0.78 and 0.90 in the ethyl ether extract and another two spots with Rf values, 0.55 and 0.68 in both ethyl acetate and ethyl acetate-methanol extracts. The chloroform extract produced a brown colored spot, Rf value 0.92, but did not fluoresce when viewed under the ultraviolet light. The above results obtained were suggestive of the presence of flavonoid compounds (Paech and Tracey, 1955). Further investigation of the individual flavonoid compounds will be undertaken later.

In the biological screening it was found that Fractions A and B possessed antibiotic activities against the first seven test organisms mentioned. However, only Fraction A showed activity against Ehrlich ascites tumor cells (EATC). These findings may be the basis for the claim of herbalists that this plant has curative properties.

Preparation of extracts using less polar solvents and further purification, identification, and biological evaluation of the active principles of this plant will be presented in the next paper.

SUMMARY

Details on the extraction, fractionation and isolation of some of the constituents of the leaves of *Coleus blumei* Benth. were presented. A long chain hydrocarbon, a sterol and triterpenoid compounds all unidentified are reported for the first time from this plant. Color reaction tests and paper chromatographic analysis showed the presence of flavonoid compounds. Preliminary biological studies indicated the presence of active constituents in the crude extracts that inhibited the growth of some microorganisms and the Ehrlich ascites tumor cells.

The claim of herbalists that this plant has curative properties may be attributed to these biological findings.

ACKNOWLEDGMENT

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IMPROVEMENT OF THE DRYING PROPERTY OF LUMBANG OIL

II. LIQUID-LIQUID SEGREGATION WITH FURFURAL

By Raul C. Obach, Violeta P. Arida, Emmanuel G. Balanquit
and Sixto A. Chua, Jr.
National Institute of Science and Technology, Manila

ONE TEXT FIGURE

ABSTRACT

The drying property of lumbang oil has been improved with a maximum increase of 15 iodine number units using liquid-liquid extraction with furfural. Studies show that the best temperature of extraction is 28°C (room temperature) while the furfural oil: volume ratio has been established at 7:1.

INTRODUCTION

In line with the study on the industrial utilization of lumbang *[Aleurites moluccana* (Linn.) Willd.] oil, research on the improvement of the drying property of the oil by solvent or liquid-liquid extraction with furfural was conducted. This was done to investigate effective applications of this locally abundant raw material which at present does not have suitable industrial use. [Obach *et al* (1972).]

Solvent fractionation is based on the principle of a liquid being incompletely miscible with another liquid at a certain temperature. Thus, when a highly selective solvent is mixed with an unsaturated oil and allowed to layer out, two portions are obtained; an upper phase consisting of the more saturated portion of oil with some solvent which is called "raffinate" and a lower layer consisting of the solvent with the most unsaturated portion of the oil called "extract." Using this principle, it is then possible to segregate an unsaturated oil into two portions of different degrees of unsaturation. [Elgin (1948) and Schwitzer (1952).]

It has been found out that the most highly selective solvent which can effectively fractionate an unsaturated oil is furfural [Elgin (1948), Mills (1952), and Schwitzer (1952)]. A number of studies has already been reported on the use of furfural for the fractionation of drying oils such as linseed or soybean oils but none has been done on lumbang oil [Beal *et al* (1961), Gloyer (1948), Goss (1948), Kenyon *et al* (1948), and Mills (1952)].

In this connection, this study was undertaken to investigate other possibilities of enhancing the drying properties of lumbang oil using furfural as the segregating solvent and to find out the maximum improvement attainable.

Part I of this series reported that it is possible to increase the iodine value of lumbang oil by at most 15 iodine number units using urea complexes [Obach *et al* (1972)]. Along this line, the application of liquid-liquid extraction with furfural is deemed highly in order since the results derived would provide basis of comparison with the urea complex process; serve as impetus for the furfural industry in the Philippines, and investigate a highly tested and exhaustively studied process for application to a local raw material [Beal *et al* (1961), Gloyer (1948), Goss (1948), Kenyon *et al* (1948), Mills (1952), and Schwitzer (1952)].

EXPERIMENTAL

Materials.—Commercial lumbang oil used in this study was supplied by the Philippine Wallboard Corporation (Nasipit Lumber Company, Inc.) and the YCO Paint Company, Phil. "Analytical Reagent" furfural was obtained from Mallinckrodt Chemical Works, St. Louis, USA.

Equipment.—ABBE Refractometer was used in the determination of the refractive indices.

Procedure.—A weighed amount of lumbang oil was shaken with measured amounts of furfural in a separatory funnel for 1 minute and allowed to stand for 30 minutes. Two portions layered out, the top portion which was composed of the oil with some amount of furfural (raffinate) and the lower layer which was composed of furfural with the unsaturated portion of the oil (extract). The two portions (extract and raffinate) were separated and individually evaporated under vacuum to distill off furfural which was subsequently collected.

The extract and raffinate portions were weighed and the yield determined. Analyses of the two portions were done using standard AOCS procedure.

RESULTS AND DISCUSSION

Liquid-liquid extraction of lumbang oil with furfural separates the former into two portions of different degrees of unsaturation.

The results are shown in Tables 1-3. The effectivity of the method is measured in terms of the relative increase in the iodine number of the resulting extract as compared with that of the original oil.

For the results shown in Table 1, small amounts of the oil sample (8 to 17 g) were used. It would seem that the best volume ratio is 3:1 (furfural:oil), which gives an improvement of 17.1 iodine number units. If the ratio exceeds 8:1 (furfural:oil), the latter becomes completely miscible with the solvent, and no separation of fractions is possible

Results of variations in extraction temperature (Table 2) using 3:1 (furfural:oil) volume ratio show that 45°C is the highest allowable temperature. Above this, furfural breaks down due to its sensitivity to temperature effects [Mills (1952), Schwitzer (1952)]. Extraction at room temperature (28°C) was found to give the highest increase in iodine value, which was 17 iodine number units.

The refractive indices show direct proportionality relationship with the iodine numbers. The more unsaturated is the bond, the higher is the refractive index, thus serving as a rough estimate of the degree of unsaturation of the oil [Bailey (1945)].

Using 3:1 (furfural:oil) volume ratio and room temperature (28°C) as temperature of extraction, multiple extractions of lumbang oil were conducted. The experimental procedure (Figure 1) involves progressive fractionation of the original lumbang oil, until a product of the highest iodine number is obtained.

From the results in Table 3, it could be seen that a furfural: oil volume ratio of 3:1 was no longer effective. The separation of the extract and raffinate as expressed in per cent yield became about 50 to 50 and the improvement in iodine number was nil. Instead of separating the more saturated portion of the oil, the original oil became simply divided into two equal portions without any significant change in iodine number (the first two extractions in Table 3). This showed that what was true for small amounts of the oil might not necessarily hold true for bigger amounts. And so, the recourse was to use the maximum volume ratio; namely, 7:1 (furfural:oil) which has also been employed by a number of workers [Mills (1952), Schwitzer (1952)]. The first extraction showed that a marked improvement of 14.25 iodine number units was achieved. This improvement was ob-

TABLE 1.—*Effect of furfural: oil solvent ratio on the degree of unsaturation of the products.*

Volume ratio furfural: oil	Weight of oil (gm)	Iodine number		Increase in iodine number (units)[1]	Refractive index (25°C)		Yield (Per cent)	
		Extract	Raffinate		Extract	Raffinate	Extract	Raffinate
2:1	17.0166	158.1	149.0	13.4	1.4778	1.4762	39.10	57.60
3:1	8.3400	161.8	151.0	17.1	1.4770	1.4752	55.90	42.90
4:1	8.3374	154.6	144.2	9.9	1.4770	1.4748	56.00	42.80
5:1	17.5722	155.3	137.4	10.6	1.4762	1.4758	37.50	60.30
6:1	17.0192	153.6	137.1	6.3	1.4757	1.4744	70.60	27.40
7:1	17.2387	148.6	139.2	1.3	1.4754	1.4740	76.90	18.40
8:1	complete miscibility of furfural and lumbang oil							

[1] Obtained by subtracting iodine number of original lumbang oil from that of the extract.

TABLE 3.—*Effect of multiple extraction at room temperature on the degree of unsaturation of the products.*

Temperature of Extraction	Weight of oil (gm)	Iodine number		Increase in iodine number (units)[1]	Refractive index (25°C)		Yield (Per cent)	
		Extract	Raffinate		Extract	Raffinate	Extract	Raffinate
28 (room temp.)	8.3400	161.8	151.0	17.1	1.4770	1.4752	55.90	42.90
35	17.3556	142.7	135.7	9.4	1.4766	1.4750	48.50	45.30
40	16.7633	136.7	131.6	3.4	1.4766	1.4745	73.00	21.82
45	17.4194	139.1	134.3	5.8	1.4763	1.4750	74.80	19.54
50	Furfural and lumbang oil become very miscible.							
60	Furfural starts to break down.							

---

[1] Obtained by subtracting iodine number of original lumbang oil from that of the extract.

TABLE 2.—*Effect of extraction temperature on the degree of unsaturation of the products using* 3:1 *(furfural:oil) volume ratio.*

Number of extractions	Volume ratio furfural oil	Weight of oil (gm)	Iodine number		Increase in iodine number (units)[1]	Refractive index (25°C)		Yield (Per cent)	
			Extract	Raffinate		Extract	Raffinate	Extract	Raffinate
1	3:1	85.68	142.5	146.8	2.90	1.4756	1.4775	57.1	41.2
2	3:1	49.74	139.4	135.6	–	1.4763	1.4758	52.6	42.3
1	7:1	94.80	153.35	135.0	14.25	1.4758	1.4744	67.4	22.1
2	7:1	51.20	153.1	142.1	14.00	1.4762	1.4754	90.8	7.4
3	7:1	34.80	153.85	140.25	14.75	1.4771	1.4780	86.7	9.5

[1] Obtained by subtracting iodine number of original lumbang oil from that of the extract.

tained by comparing the iodine number of the extract with that of the original lumbang oil. The increase in iodine value for the second and third extractions was 14.00 and 14.75, respectively.

Results obtained herein show that the maximum improvement in unsaturation attainable was about 14 to 15 iodine number units. This is the ceiling point for the "segregation" process of lumbang oil beyond which no improvement is possible. These findings agree consistently with the results reported in a previous paper of this series [Obach *et al* (1972)].

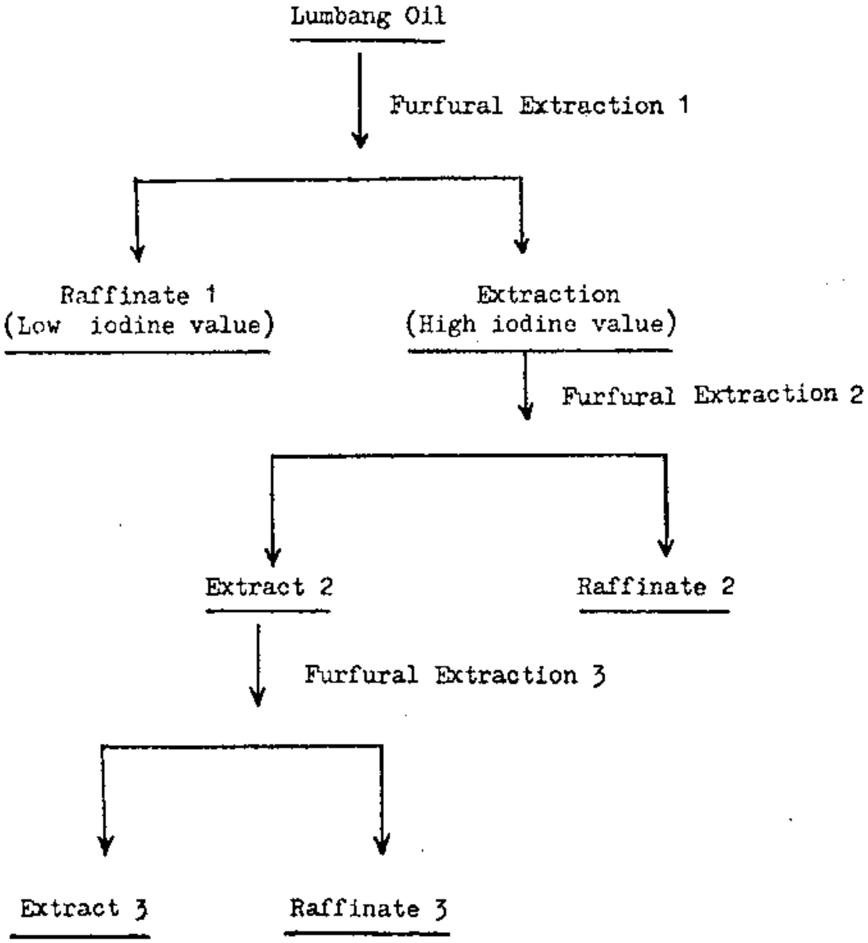

FIG. 1. Multiple extraction scheme of lumbang oil by liquid-liquid extraction with furfural.

CONCLUSION

This study shows that it is possible to increase the iodine value of lumbang oil by 15 units using liquid-liquid extraction with furfural. However, the development of this process for industrial application in the Philippines would ultimately depend on the following factors:

1. The industrial advantages that could be derived from lumbang oil with an improvement of 15 iodine number units, considering the economics of large scale production.

2. The uses for which the raffinate (10 to 15 per cent of the starting oil) could be intended, possibly as food grade industrial chemical.

3. Recovery of furfural — Using this process, 97 to 98 per cent of the original volume of furfural used could be recovered. This could be purified and recycled to cut down production expenses. Furthermore, the extent of furfural recovery could be improved considerably especially with the application of continuous processes.

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RESPONSE OF COCONUT TO NPK FERTILIZATION AT DAVAO

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ONE PLATE AND ONE TEXT FIGURE

ABSTRACT

Various levels of NPK fertilization were tested on 8-year-old coconut trees for a period of 5 years in an area where the relief ranges from nearly level to undulating. The soil is Tugbok clay with a pH of 6.0, 2.4-per cent O.M., 48-ppm available P_2O_5, and 300-ppm exchangeable K_2O.

Results show that the fertilized trees gave a yearly increase of 23- to 55-per cent nut production over the control (unfertilized). The best treatment (0.3-kg N and 1.0-kg K_2O per tree) gave an increase of 32- to 80-per cent nut production annually commencing on the second year after the first application. This treatment is recommended for coconut plantations where the agronomic and climatic conditions are similar to those obtaining at the Research Center in Davao City.

INTRODUCTION

The Philippine government in its desire to rehabilitate the coconut industry sought financial and technical assistance from the recently terminated joint project of the Bureau of Soils — United Nations Soil Fertility Survey and Research Project of the Philippines. To this effect the UN Special Fund and the Bureau of Soils, in collaboration with the Bureau of Plant Industry deemed it proper to carry on this project until 1969. In 1970, the Philippine Coconut Research Institute (PHILCORIN) continued the gathering of data.

The main objective of this project is to study the response of coconut to the application of nitrogen, phosphorus and potassium.

REVIEW OF LITERATURE

Lockard *et al* (1966) in their "Planting and Preliminary Fertilizer Recommendation in the Philippines," recommended the annual application of 2 kilos of muriate of potash per tree, to low-yielders (less than 60 nuts per tree). It was further recommended that during the first 2 years, 1 kilo be applied every 6 months after which 2 kilos can be applied once a year.

Krishna Marar and Pandalai (1961) claimed that nitrogen and potash show different effects on yield and quality. Nitrogen has a quick effect on yield while potash has a delayed effect. Nitrogen has an adverse effect on the nut characters whereas potash has very beneficial effects. To get optimum benefits, a balanced application of nitrogen and potash is very necessary. They concluded that for loamy soils, the optimum requirement of nitrogen is about 1 pound per tree per year, that of potash about 1.2 pounds while the response of the palms to phosphoric acid has been very little.

Salgado (1947, 1952), as cited by Menon and Pandalai (1958), has shown that potash affects earliness of bearing of the palms in Ceylon. Palms receiving potash were observed to fruit in the fifth year of planting while palms receiving no fertilizer bore fruit in 8 years. It was also observed that nitrogen caused an 8 per cent increase in copra yield while potash gave an increase of 25 to 35 per cent.

Patel and Nair (1936) have observed that potash can reduce the incidence of shoot-rot attack and this has been attributed to the potash moiety of the nutrients which increase the resistance of the protoplasm to pathogenic organisms.

Halliday and Sylvester (1954), commenting upon phosphorous manuring of coconuts in Ceylon, stated that at Bandirippwa, where there was adequate reserve of phosphorous, there was no response to phosphorous application. Experiments in the poor laterite soils of Veyanagoda and Ahangama with low phosphorous resources on the other hand, gave spectacular responses to application of 0.60 pounds of P_2O_5 per palm every other year. Response to phosphoric acid was observed also at Ratmalagara where the soil is reported to be loamy. As observed in our Center, highly significant response was seen at the rate of 1.0 pound per tree once a year. Salgado (1955) in an attempt to explain the difference of phosphoric acid requirements at Bandirippwa and Ratmalagara, came to the conclusion that the levels of available phosphate as determined by the citric acid or Truog methods have little prediction value as a basis for assessing the need for phosphate manuring. In this connection, he postulates the need of organic phosphorus in the nutrition of the coconut palms.

Some results of the different manurial experiments done in India, Ceylon, the Philippines, Malaya, and Trinidad as summarized by Menon (1960) are as follows:

1. There is a general response to the application of potash and nitrogen, while response to phosphoric acid is seen only in restricted situation.

2. A minimum of three years appears to be a reasonable period for the first response of the trees fertilized.

3. There is general agreement that initially high yielders do not respond as much to manuring as the low and medium yielders. This indicates the possibility of selective manuring of the trees especially when the prices of fertilizers are high.

4. Regarding the effect of manures on characters other than the yield, Salgado (1946) as cited by Menon and Pandalai (1958) is of the opinion that (*a*) nitrogen has a beneficial effect on female flower production, potash has little effect and phosphoric acid, a definite depressing effect; and (*b*) on copra content, nitrogen has an adverse effect as a high number of nuts are required to produce copra, potash has a very beneficial effect while phosphoric acid has no effect. According to Child and Salgado (1941), the oil and protein contents of copra are not influenced by different nutrients. Patel (1938) as cited by Menon and Pandalai (1958) reported of having obtained the highest quality copra and oil from plots receiving complete manure containing NPK.

MATERIALS AND METHODS

This experiment was carried out on 8-year-old trees in San Gabriel, Mintal; Davao City where the PHILCORIN Research Center is located. The relief ranges from nearly level to undulating. Surface drainage is good and internal drainage is fair to good.

The soil type is Tugbok clay with a pH value of 6.0, 2.4-per cent organic matter, 48-ppm available P_2O_5, and 300 ppm exchangeable K_2O. Surface soil is about 30 to 35 cm deep.

Rainfall for a 10-year average shows a peak of 17.5 inches during May declining in the succeeding 3 months. Dry months are December, January and February with a monthly average of 5 to 6 inches.

Temperature for a 10-year period ranged from 71° to 82°F.

The experimental design is a randomized complete block of seven treatments replicated three times.

Ring weeding and inter-row harrowing were done as often as necessary but not less than 6 times during 1 year.

Each plot or treatment were composed of nine trees and between each plot are guard rows of trees numbering 200 in all.

Every year fertilizer were applied accordingly to the seven treatments ($N_0P_0K_0$, $N_1P_0K_0$, $N_1P_0K_1$, $N_1P_0K_2$, $N_1P_1K_0$, $N_1P_1K_1$, $N_1P_1K_2$) in the form of ammonium sulphate as source for N, superphosphate for P_2O_5 and potassium chloride as source of K_2O. The rates were as follows:

N_0 = No nitrogen
N_1 = 0.3 kg N/tree
P_0 = none
P_1 = 0.6 kg P_2O_5kg/tree

K_0 = none
K_1 = 0.5 kg K_2O/tree
K_2 = 1.0 kg K_2O/tree

RESULTS AND DISCUSSION

Two years after fertilizer application response of plants became evident especially those treated with nitrogen and potassium fertilizers. The leaves were denser and dark green in color, petioles and leaf blades were larger, nuts were larger and ripening hastened.

Table 1 shows the yearly effect of the various treatments on nut production. The fertilized trees gave an increase of 11.1 nuts per tree in 1966 and 27.5 nuts in 1970 over the non-

TABLE 1.—*Average number of nuts per tree per year.*

N P K treatment	1965	1966	1967	1968	1969	1970
N - P_2O_5 - K_2O						
(0-0-0)	35.7	51.0	45.9	76.8	66.3	88.9
(1-0-0)	48.4	47.0	52.6	84.2	92.0	112.2
(1-0-1)	38.5	46.9	50.0	81.0	91.0	102.7
(1-0-2)	40.4	52.9	69.8	124.2	119.0	135.0
(1-1-0)	44.0	52.9	48.0	85.2	95.6	109.3
(1-1-1)	38.5	70.4	69.1	103.2	110.3	127.0
(1-1-2)	36.5	67.9	59.4	93.6	107.3	113.4
Mean		60.5	56.4	92.6	97.3	112.5

fertilized trees. This trend of nut production is shown in Fig. 1. The best treatment (1 - 0 - 2) or 0.3 kg of N as ammonium sulphate containing 20-per cent N and 1.0 kg of K_2O per tree

FIG. 1. Response of coconut to fertilizer at PHILCORIN, Davao City (UNDP) 1965-1970

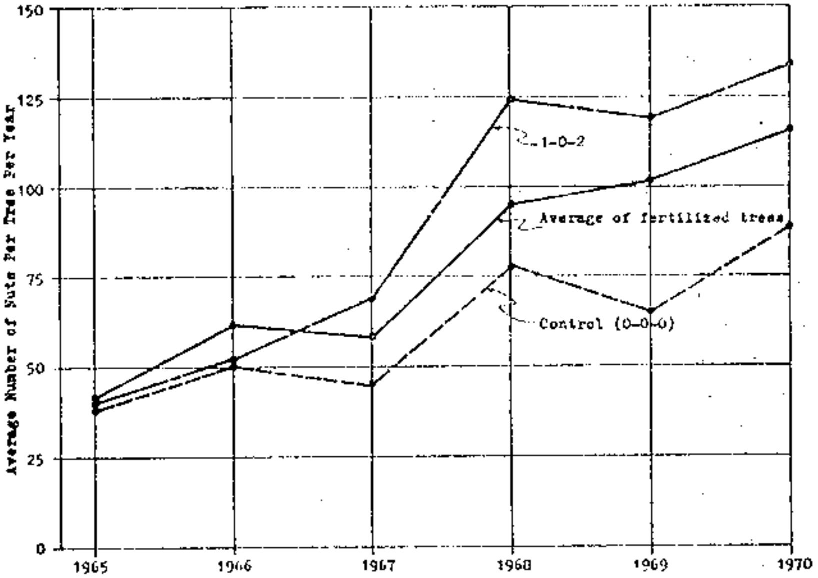

TABLE 2.—*Yield of copra in kg per tree.*

N P K treatment	1965	1966	1967	1968	1969	1970
$N - P_2O_5 - K_2O$						
(0-0-0)	6.83	6.67	6.37	10.10	9.06	11.87
(1-0-0)	8.00	6.00	7.60	10.97	12.07	15.72
(1-0-1)	6.53	7.47	11.03	16.37	19.73	20.37
(1-0-2)	10.10	15.73	14.63	27.70	29.90	29.50
(1-1-0)	5.73	6.43	6.13	10.50	12.47	12.81
(1-1-1)	6.90	11.00	12.63	17.73	21.23	22.74
(1-1-2)	7.40	13.33	15.33	20.30	24.37	24.50
Mean	7.36	9.52	10.53	16.34	18.40	19.64

Significant difference:					
Per cent					
5	4.09	6.67	4.93	5.89	6.87
1	5.72	9.33	6.89	8.24	11.46
C.V. (per cent)	24.2	35.9	17.0	18.0	19.8

as potassium chloride containing 60 per cent K_2O gave an increase of 37 nuts per tree in 1966 and 46.1 nuts in 1970 over the control. The highest yield increment for this treatment was 52.7 nuts per tree in 1969 or 79 per cent more than the tree which did not receive any fertilizer.

It may be noted that the age of coconuts at which the experiment was started was 5 years old or the age at which coconut starts bearing. From hereon, fruit production would increase gradually up to a certain time, hence, the noted increase per tree from 35.7 nuts in 1965 to 88.9 nuts in 1970 was 149 per cent even without the application of fertilizers. On the other hand, the application of the right kind and amount of fertilizers are efficient than the effect of age on the yield of nuts. By comparing the yield of nuts of treatment No. 4 (1 - 0 - 2) and the nonfertilized trees (Treatment No. 1), the yield increment over the control are 72 per cent in 1966, 52 per cent, 62 per cent, 79 per cent, and 52 per cent in the subsequent years.

Plate 1, figs. 1 and 2 shows a comparison between the tree fertilized with 1.5 kg ammonium sulphate plus 1.66 kg potassium chloride and the unfertilized trees.

Table 2 shows the effect of NPK fertilization on the yield of copra. Among the seven fertilizer treatments studied, treatment No. 4 again seems to be the most decisive combination of fertilizers for increasing the yield of copra which is derived from the increase in number and size of nuts and the weight of the kernel. The yield trend in six successive years where shown in Table 2 whereby response to this treatment, already apparent in 1966, remain in the same order of magnitude in the subsequent years. It gave the highest yield of copra among the seven fertilizer treatments. The noted increase in yield for these treatments are 133 per cent in 1966, 129.7 per cent in 1967, 172.3 per cent in 1968, 230.1 per cent in 1969, and 72.7 per cent in 1970 over the control.

CONCLUSION

For coconut plantations where agronomic and climatic conditions are similar to Davao City, a combination of 0.3-kg N (1.5-kg ammonium sulfate, 20-per cent N) and 1.0-kg K_2O (1.6-kg muriate of potash, 60-per cent K_2O) per tree per year is recommended.

ACKNOWLEDGMENT

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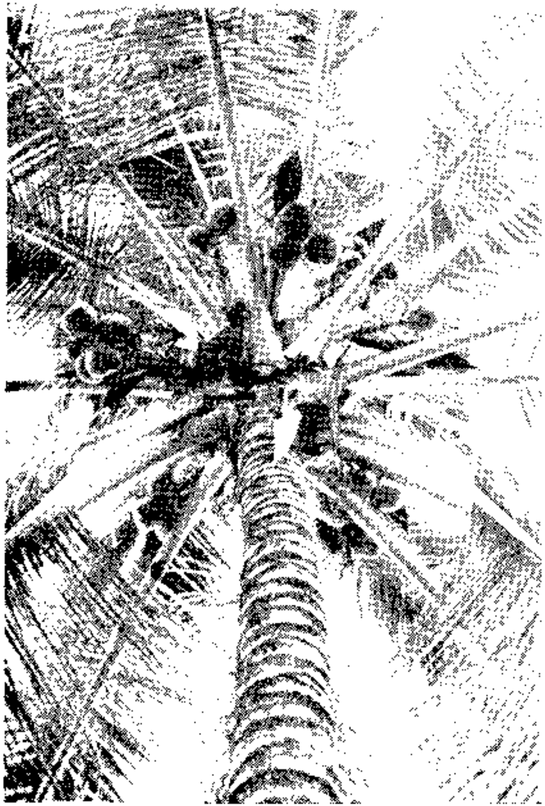
ILLUSTRATION

PLATE 1

FIG. 1. Unfertilized coconut tree. NPK fertilizer experiment, PHILCORIN Research Center, San Gabriel, Davao City.

2. A coconut tree fertilized with 0.3-kg N and 1.0 kg per tree. NPK fertilizer experiment, PHILCORIN Research Center, San Gabriel, Davao City.



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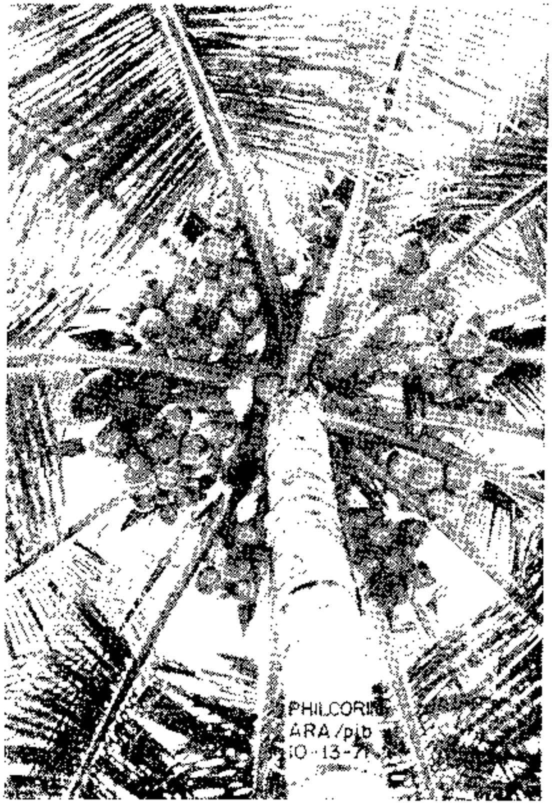

2

PLATE I

STUDIES ON SOLVENT EXTRACTION OF RESIDUAL OIL FROM WET COCONUT MEAL USING ISOPROPANOL

By A. L. Gonzales, E. F. Buccat, T. R. Claudio, N. M. Bueser,
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THREE TEXT FIGURES

ABSTRACT

The applicability of isopropanol as an extractant for the residual water and oil in the wet coconut meal obtained by the wet processing of coconut meat was investigated. The effect of four variables; namely, meal to solvent ratio, number of extractions, time and temperature of extraction on the efficiency of oil recovery was determined. Results of the factorial extraction experiments conducted indicate that a maximum amount of oil could be extracted from the wet meal under a set of conditions involving the combination of the extreme values of the four parameters considered in this experiment.

INTRODUCTION

The processing of oilseeds has been a vital industry for the past several years. During this time both the equipment and the methods employed have undergone practically continuous evolution from primitive towards modern machinery practices.

Although many processes are used to recover oil from seeds, the three more common procedures are hydraulic pressing, expeller pressing, and solvent extraction. All three are used widely and frequently in combination. For the processing of a number of oilseeds, continuous presses such as the Anderson expeller, the French screw press and the Hander expeller have replaced the batch hydraulic equipment. These expellers, used the world over on a variety of oleaginous materials, have proved quite successful because of lower operating costs and because they require very little hand labor. In some countries, however, their chief function is to forepress seeds containing a high percentage of oil, to reduce the oil content of these seeds sufficiently to permit the use of solvent extraction for "finishing" the process.

The advantages of using solvents to extract oil from seeds have long been recognized. Solvent extraction is relatively the most efficient method of processing oilseeds and other materials of low oil content; containing not more than 30 per cent of oil

[Mattil *et al* (1964)]. It reduces the residual oil in the extracted cake to as low as 1 per cent.

But in spite of the comparatively high recovery of oil, solvent extraction suffers a number of disadvantages which have caused oil millers to proceed slowly in its adoption. Seeds containing a large amount of oil cannot be formed readily into sufficiently rugged flakes or into any other shape which lends itself to rapid extraction of the oil without disintegrating. It is for this reason that many operators forepress these raw materials which contain high percentages of oil.

Another disadvantage of the solvent extraction process is its initially high cost. An estimate indicated that extraction facilities cost three times more than the equivalent expeller equipment. The higher yield of oil obtained by extraction, however, much more justifies any slight disadvantage which the solvent method might have with respect to operating cost.

In general, paraffin hydrocarbons of the proper volatility have been found to be the most efficient solvents for removing the oil along with a minimum of nonoil materials and subsequently lending themselves to easy separation from the crude oil by distillation. These solvents, however, are inflammable so that considerable thought has been given to the use of noninflammable solvents, principally the chlorinated hydrocarbons. Trichloroethylene and its homologs are now used in many small extractors processing soybeans and a variety of oilseeds. These solvents possess disadvantageous characteristics, however, including a relatively high cost and a tendency to dissolve even nonglyceride materials [Mattil *et al* (1964)].

The alcohols, ethanol, and isopropanol have also been considered as solvents. They yield a meal of better flavor and color than does extraction with hexane. Studies on the extraction of cotton-seed oil with isopropanol and ethanol indicated that complete miscibility of isopropanol with the oil can be attained at the temperature below the solvents' normal boiling point at moisture contents as high as 10 per cent whereas ethanol can tolerate only about 1.5 per cent [Magne and Skan (1953)]. Carangal and Banzon (1949) made use of ethanol to extract the oil from sun-dried coconut shreds. To date, however, there are no known processes on the direct solvent extraction of wet coconut meal.

Filtration extraction, an improved process of solvent extraction was originally developed for cotton-seed [Magne and Skan

(1953)] and has been successfully applied to sesame [Graci *et al* (1956)], peanut [Smith (1971)], soybean [Gastrock (1956)], coconut [Aliwalas and Buccat (1967)], and other oil bearing materials.

The development and improvement of coconut processing had been the subject of a number of scientific and technological researches [Aliwalas *et al* (1969), Andaya *et al* (1961), Bhatia (1963), Birosel *et al* (1968), Birosel and Floro (1955), Bousser and Bermer (1969), Carangal and Banzon (1949), and Paper (1958)]. The most recent development in this field has been known by a lot of terms, among which are: wet processing, aqueous extraction, wet rendering and fresh fruit processing. All these methods are essentially similar; in that fresh coconut meat rather than the conventional sun-dried meat, or copra, is utilized as the starting material.

In 1969, a process for the production of canned gata was developed in the FRL of the NIST [Gonzales *et al* (1971)] making use of this wet method. Essentially, the process consists of subjecting the ground coconut meat to a series of three expressions in the Hander Expeller adding tap water in the amount of ¼ the weight of the coconut meat for the 2nd and 3rd extractions. The meal obtained from this 3-stage expressions still retains 25- to 28-per cent oil and 45-per cent water. Because of this, the meal cannot possibly resist spoilage even for very short periods of storage. Its high moisture content tends to accelerate hydrolytic decomposition of the proteins and oil present in the system. If by some special process, only the moisture is removed, the dried meal with its high oil content easily becomes rancid and has very limited application in food formulations requiring low oil levels. In order to improve its keeping quality and to promote its utilization, wet coconut meal is subjected to this study with the objective of establishing the best conditions for reducing both its oil and moisture contents. This study seeks to determine the suitability of alcohols for use as solvents of the residual oil and water from wet coconut meal and to establish the optimum extraction conditions.

MATERIALS AND METHODS

The wet coconut meal used in this study was obtained from the NIST pilot plant processing canned gata by the method shown in Figure 1.

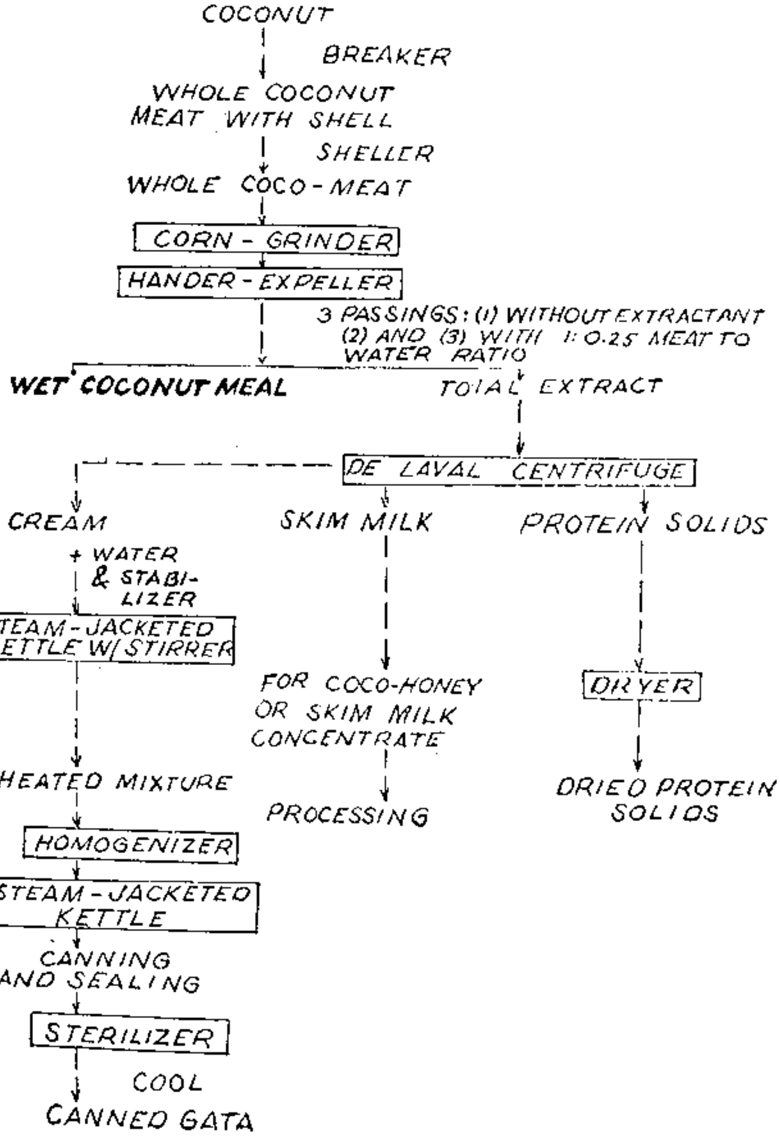

FIG. 1. Flow-diagram for the pilot plant processing of canned gata.

Technical grade isopropanol and 95-per cent ethanol were the solvents used to extract the residual oil and water from the wet meal.

A weighed quantity of the wet meal was macerated in a measured volume of solvent with the aid of a motor-driven stirrer. The extraction was allowed to proceed for a predetermined length of time, maintaining a certain temperature. The miscella was then filtered off and the solvent-extracted meal was dried over low heat to evaporate off the remaining solvent. Finally, the desolventized dried meal was weighed.

The meal before and after solvent extraction was analyzed for moisture, oil and protein contents. The amount of moisture in the meal was determined by heating a representative sample of the material at 130°C in a forced-draft oven for one hour. Oil content was determined by the rapid method of Cruz *et al* (Unpublished report) while nitrogen determination was done by the micro-Kjeldahl method [AOAC (1970)]. The protein content was estimated by multiplying the per cent nitrogen so obtained by the factor 6.25.

The per cent efficiency of extraction of the residual oil from the wet coconut meal was calculated as follows:

$$\text{Efficiency of oil extraction (per cent)} = \frac{\text{Amount of oil extracted}}{\text{Total amount of oil extractable from the meal}} \times 100$$

The effect of four varying factors on the efficiency of extraction of the residual oil from the wet coconut meal was considered in this study. These factors are: (*a*) meal to solvent ratio, (*b*) extraction temperature, (*c*) number of extractions, and (*d*) extraction time.

A 3 × 3 × 2 × 3 factorial experiment in a randomized block design was used. The different levels of the four factors are as follows: (*a*) meal to solvent ratios of 1:10, 1:20, and 1:30, (*b*) extraction temperatures of 30°C, 50°C, and 60°C, (*c*) extraction times of 20 and 40 minutes, and (*d*) number of extraction of 1, 3, and 5 extractions.

The simple random sampling method was used to determine the treatment combinations to be conducted at a given time. Two replications were made for each of the treatment combinations. The analysis of variance technique (ANOVA) was used in the statistical evaluation of the results of the extraction experiments.

RESULTS AND DISCUSSION

The chemical analysis of wet coconut meal obtained from different batches is shown in Table 1.

Preliminary experiments were conducted to compare the effectiveness of isopropanol and ethanol as extractants of the

TABLE 1.—*Chemical composition of different batches of wet coconut meal.*

Trials No.	Chemical analyses				
	Oil			Protein (N x 6.25)	
	Moisture	Wet basis	Dry basis	Wet basis	Dry basis
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	47.96	25.23	48.57	4.77	9.16
2	48.79	24.88	48.58	3.83	7.48
3	47.67	25.85	49.39	3.54	6.76
4	44.12	27.91	49.94	3.61	6.47
5	47.27	27.54	52.32	3.19	6.05
Average	47.16	26.29	49.76	3.79	7.18

residual oil from the wet coconut meal. The results are shown in Figure 2. Isopropanol extracted 83 per cent of the original oil from the starting material in an experiment using a meal to solvent ratio of 1:20 while ethanol removed only 76 per cent. Similarly, for other trials making use of varying ratios of meal to solvent, isopropanol gave a better oil extraction efficiency. These results indicate that under identical conditions, isopropanol is better than ethanol as an oil extractant for wet coconut meal.

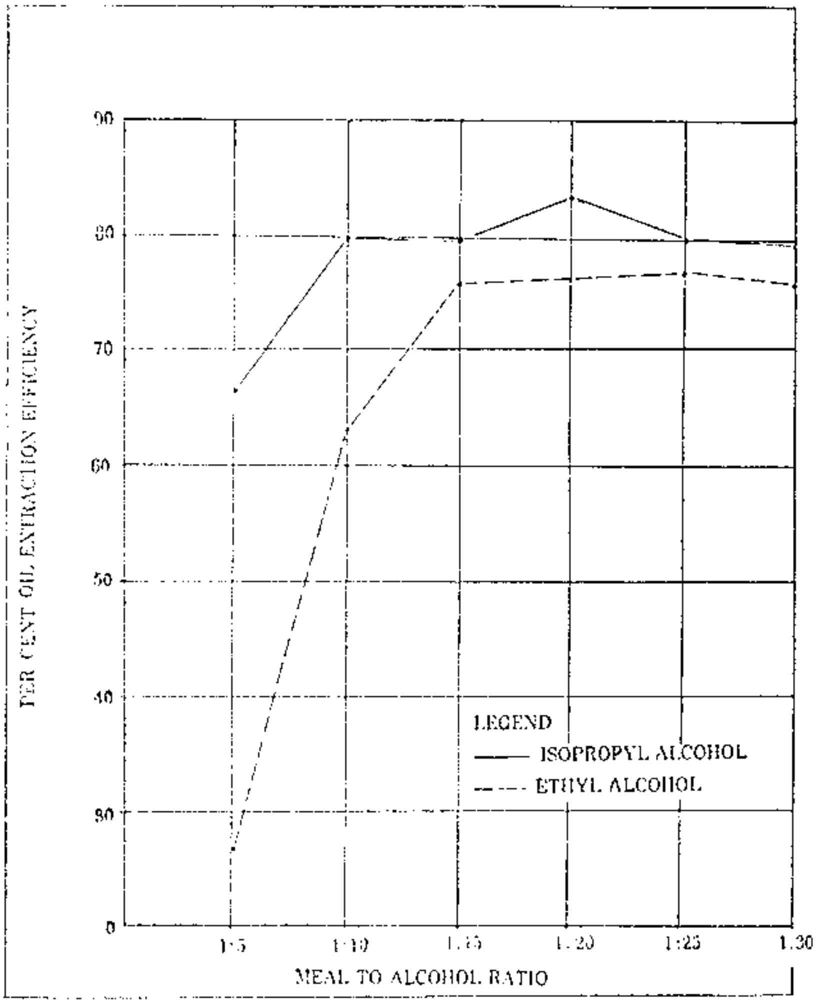

Fig. 2. Graph showing the effect of solvent on the efficiency of extraction of oil from wet coconut meal.

This variation in the oil extraction efficiency reflects the differential solubility of the oil in the two solvents. The effectiveness of isopropanol as a solvent may be attributed to its longer carbon chain which forms stronger van der Waals forces of interaction with the hydrocarbon portion of the oil molecules.

From the above results and based on economic considerations, it was decided that this solvent be used as the extractant in the succeeding extraction experiments.

Table 2 shows the effect of the four variables considered in this study on the efficiency of extraction of oil from wet coconut meal using isopropanol while Table 3 gives the ANOVA table for the extraction efficiency data gathered from the factorial experiment. Based on the computed F-values for the four different factors, all these factors were found to have highly significant effects on the oil extraction efficiency at 5-per cent level of significance. Comparison of the computed F-values indicates that of the four main factors, the number of extractions seems to have the greatest bearing in affecting the efficiency of oil removal from the meal while the period of extraction, the least.

Statistical information further reveals that second order interactions are insignificant so that any two factors act independently of the other two factors in affecting the efficiency of oil extraction. There exists, however, slight significance in third order interactions which indicates that any three factors are dependent on the fourth variable in affecting the oil extraction efficiency.

Figure 3 indicates that for all the different treatment combinations involved in the study, the amount of residual water extracted from the meal is the same. The suitability of isopropanol as extractant for water in the meal regardless of the extraction condition may be due solely to the nature of the isopropanol molecule. Hydrogen bonds are formed between the hydroxyl groups of the alcohol molecules and the water molecules in the meal thus facilitating the removal of the water. The use of isopropanol then do away with the usual predrying of the wet meal prior to its solvent extraction since simultaneous extraction of the residual oil and water is possible with this solvent.

This study has thus established that a maximum amount of oil may be removed from the wet coconut meal using a series of 5 extractions on the material of a 1:30 meal to solvent ratio at

TABLE 2.—*Percentage efficiency of extraction of oil from wet coconut meal using isopropyl alcohol as solvent and the corresponding effect of the variables considered.*

Time of extraction	Meal to alcohol ratio	1:10			1:20			1:30		
Time Min.	No. of extractions Temp. °C	1	3	5	1	3	5	1	3	5
20	Room temp.	38.31	48.86	57.23	63.49	64.54	71.92	65.28	56.94	88.91
	50	59.62	80.43	71.06	64.81	69.25	84.50	75.30	78.46	85.16
	60	53.90	72.37	68.74	59.91	71.01	80.64	71.88	75.70	90.22
40	Room temp.	40.40	75.93	72.62	55.16	62.03	71.73	55.02	72.62	75.12
	50	69.41	76.69	79.16	67.32	79.83	90.29	79.26	88.15	89.11
	60	57.81	61.87	76.43	77.09	81.62	85.84	79.41	85.34	85.55

TABLE 3.—*Anova table.*

Sources of variation	Degrees of freedom (df)	SS	MSS=SS/df	F_c	F_t
Meal to solvent ratio (M)	2	1,571.5271	785.7635	31.90	8.65
Number of extractions (N)	2	2,371.2616	1,185.6308	48.14	8.65
Time (T)	1	263.1843	283.1843	19.36	5.32
Temperature (t)	2	1,962.8358	981.4179	39.84	8.65
Interactions					
M x t	4	109.7919	27.4480	1.11	3.84
M x N	4	232.8343	58.1086	2.35	3.84
M x T	2	35.8936	17.9468	0.72	8.65
N x T	2	51.0892	25.5446	1.04	8.65
N x t	4	75.8564	18.9641	0.77	3.84
T x t	2	17.3142	8.6571	0.35	8.65
M x N x T	4	200.6965	50.1741	2.04	3.84
T x t x M	4	359.1874	89.7969	3.64	3.84
T x t x N	4	271.9122	67.9780	2.76	3.84
M x N x t	8	245.7356	30.7170	1.25	3.84
Error	8	197.0575	24.62969		
Total	53	7,986.1576			

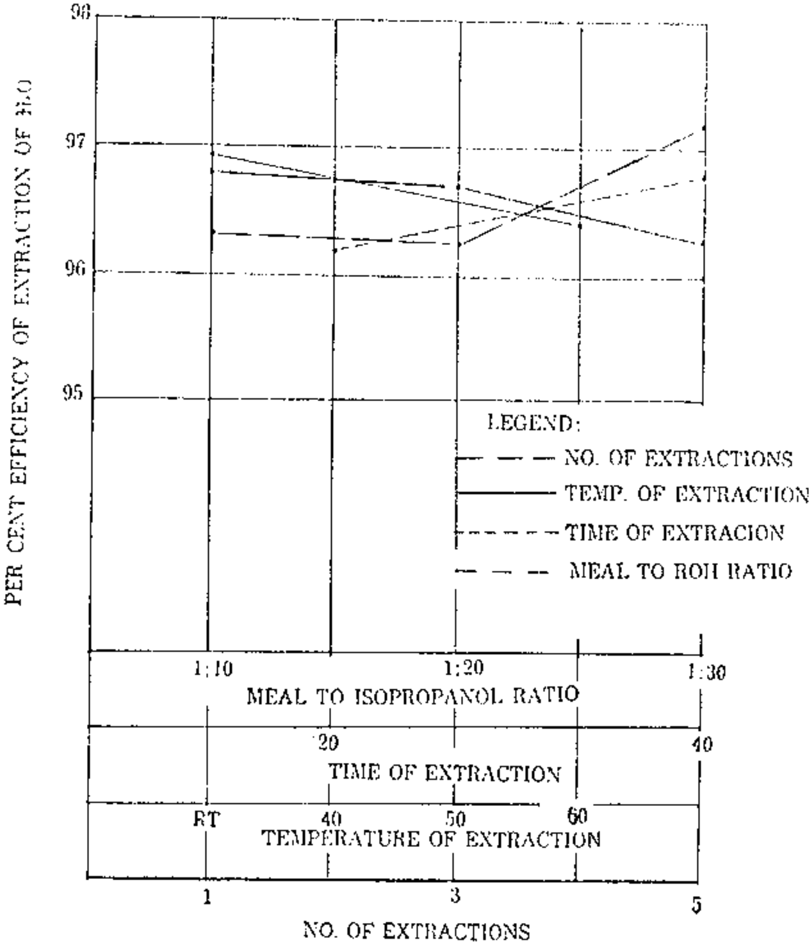

FIG. 3. Graph showing the effect of the different variables on the efficiency of extraction of water from wet expeller-pressed coconut meal.

TABLE 4.—*Percentage efficiency of extraction of water and oil from wet coconut meal using the established conditions for maximum extraction.*

Samples	Weight of materials	Chemical analyses *			Extraction efficiency	
		Moisture	Oil	Protein (Nx 6.25)	Water	Oil
	g	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Wet coconut meal	20.00	47.86	28.43	3.57		
Solvent extracted meal	6.01	3.59	14.03	14.52	97.73	85.15

* "as received" basis.

an extraction temperature of 60°C and an extraction time of 40 minutes.

Under the same set of conditions, 97 per cent of the original water of the wet meal may be extracted. The extraction efficiencies of oil and water from the wet meal using the established conditions is shown in Table 4.

This finding, however, does not necessarily preclude that such set of conditions is the most economical nor the most applicable for commercial scale extraction of oil from wet coconut meal. A bench-scale study and later a pilot plant-scale study are recommended to follow the laboratory phase of the research in order to establish the necessary data for commercial-scale operations.

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PREPARATION OF TRILAURIN: CHROMATOGRAPHIC STUDY OF REESTERIFICATION OF METHYL LAURATE WITH GLYCEROL

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TWO TEXT FIGURES

ABSTRACT

Conditions most favorable for the laboratory preparation of trilaurin by reesterification of methyl laurate with glycerol in the presence of a catalyst were investigated and established. Chromatographic methods were employed to follow the rate of reaction by direct analysis of the trilaurin present in the reaction mixture.

INTRODUCTION

The production of monoesters from coconut oil (almost 50 per cent of which is lauric acid) by alcoholysis was studied by A. R. Aliwalas *et al* (1969). These monoesters are suitable starting materials for various products, the preparation of triglycerides being one of its specific applications. Triglycerides find wide industrial use, such as the manufacture of "high ratio" shortenings, oil modified resins and as intermediates for the preparation of the so-called tailor-made fatty products (Gros and Feuge, 1949; Feuge and Bailey, 1946). Medium chain triglycerides (caproic-lauric) are used in medicine for the treatment of malabsorption syndromes and allied clinical disorders, as carriers for therapeutic agents such as vitamins and antibiotics. These triglycerides and modifications of its esters have other special medicinal and nutritional uses for future consideration (Babayan, 1968; Barsky and Babayan, 1961).

The preparation of triglycerides from monoesters can be accomplished by: (*1*) Indirect method—this includes those in which the monoesters are first converted to free acids, acid anhydrides or acid chlorides before being reacted with glycerol and (*2*) Direct method—the processes involve only alcoholysis or reesterification with glycerol and ester-ester interchange. The direct method is more desirable from the standpoint of cost and simplicity. (Gros and Feuge, 1949.)

Reesterification reaction with glycerol is best carried out at an elevated temperature while maintaining a vacuum to permit the escape of water vapor and a partial condenser to condense and return glycerol vapors to the vessel. (Bailey, 1951.) In order to obtain a product consisting substantially of triglycerides, the ester or fatty acid must be present in excess of the equivalent amount of glycerol (Bailey, 1951; Hilditch, 1949; Mills, 1952). If glycerol is present in excess, the reaction product will consist mainly of monoglycerides (Choudhury, 1962). The reaction is considerably accelerated by the use of a suitable catalyst. Bhattachraya and Hilditch recommend the use of aromatic sulfonic acids, but this causes a certain degree of darkening aside from the difficulty of obtaining this catalyst. A number of patents specify various metals and metal oxides including compounds of zinc and tin (Ralston, 1948). Kroemer and Bailey tested a number of metal compounds as catalysts for the esterification of glycerol with peanut oil fatty acids. The most active were tin and zinc chlorides but they had a tendency to form metallic soaps with the FFA and glycerol (Gros and Feuge, 1949; Bailey, 1951). Several publications on the preparation of technical glycerides cited the use of NaOH as catalyst (Choudhury, 1962; Feuge and Gros, 1950). The preliminary runs we conducted showed that the use of NaOH as catalyst was effective. Because of its availability and low cost, this was used in subsequent experiments.

Analysis of the reaction mixture proved to be a problem because there is no direct chemical procedure for the determination of total mono-, di- and triglycerides in the presence of each other, although the $\propto$ monoglyceride content can be determined by the AOCS method (1958). They may be separated by batchwise and countercurrent extractions, by urea adduct formation, or by molecular distillation. These methods, however, require expensive equipment and consume considerable time for each determination. Several investigations have shown that column chromatography affords a simple method for the quantitative separation and determination of mono-, di-, and triglyceride concentrates (Distler, 1965; Monick and Treybal, 1956; Quinlin and Weiser, 1958). The number of OH groups in the glyceride affects tenacity of adsorption while chain length, position and unsaturation of the fatty acid group have a minor effect on adsorption.

For purification purposes, selective liquid-liquid extraction techniques are adoptable to mixtures of mono-, di-, and triglycerides based on the fact that they differ in molecular weight and number of polar groups (Monick and Treybal, 1956). Crude trilaurin can be purified by extraction with aqueous ethanol from its solution in petroleum ether and finally by crystallization from alcohol. This phase is the subject of another investigation.

In view of a desire to produce locally, chemical derivatives of industrial importance from coconut oil or its intermediates which would command a much higher market value, diversify exportable products and eventually help attain market stability for the coconut industry in the Philippines, this study was undertaken.

The aim is to establish the conditions most favorable for the production of trilaurin by reesterification of methyl laurate with glycerol. The parameters involved are: (*1*) temperature, (*2*) extent of reaction time, (*3*) proportion of methyl laurate and glycerol, (*4*) nature and concentration of catalyst, and (*5*) effect of pressure.

The direct determination of the amount of trilaurin in the reaction mixture was investigated using column chromatography based on the procedure of Distler and Bauer (1965) with the aid of thin-layer chromatography (Stahl, 1965).

MATERIALS AND METHODS

Materials.—Methyl laurate (N_D^{25} 1.4304) was prepared from coconut oil in the NIST Pilot Plant, Manila, Philippines and redistilled in the laboratory.

Glycerine, technical grade was redistilled in the laboratory, Sodium Hydroxide pellets, A. R. (97.0 per cent) was obtained from Mallinckrodt Chemical Works, St. Louis, U.S.A.

Solvents for analysis: Benzene, carbon tetrachloride, chloroform, petroleum ether, ether-all chromatographic grades, Riedel-De Haen, AG Seelze, Hannover, Germany.

Silica gel — Kieselgel S for column chromatography (CC)
— Kieselgel G nach Stahl for thin-layer chromatography (TLC)

Iodine crystals, U.S.P.

Apparatus.—Reaction was carried out in a three-necked flask fitted with a thermometer, nitrogen inlet and an air condenser. A water-cooled condenser was connected by means of a still head (with thermometer) to the air condenser to collect the methyl alcohol vapors. This set-up was provided with a magnetic stirrer and heat was supplied by a heating mantle with a regulator to control the temperature.

Burets improvised with reservoir bulbs were used as columns for chromatography.

Microscopic slides were used as TLC plates.

Procedure.—Preparation of sample: Methyl laurate and glycerol in the presence of the catalyst were allowed to react for several hours under a nitrogen blanket. After constant temperature has been attained, samples were withdrawn periodically for analysis of trilaurin.

Method of analysis.—The weighed sample (1.00 g) was dissolved in chloroform (15 ml) and passed into a silica gel packed column. Trilaurin and any unreacted methyl laurate were eluted with benzene (300 ml). Eluates were collected in fractions and each was tested for the presence of trilaurin and methyl laurate by TLC using benzene-ethyl ether (8:2) as developer. After complete elution, all fractions containing these components were mixed together and the solvent removed by means of a Rotavapor. The residue was dried in a vacuum oven at 60°C, weighed and calculated as per cent of the original sample.

If methyl laurate is present in the residue as detected by TLC using carbon tetrachloride as developing solvent, analysis proceeded as follows:

The residue from the benzene eluate dissolved in chloroform (15 ml) was passed through another silica gel packed column. Methyl laurate was eluted with carbon tetrachloride (300 ml) and trilaurin subsequently eluted with benzene (300 ml). Completeness of resolution and elution was detected by TLC.

For TLC, microscope slides coated with silica gel slurried in chloroform were used. The samples were applied as spots to the air dried TLC plates, allowed to develop in the suitable solvent, air dried and visualized by brief exposure to iodine vapor.

Variation of reaction parameters.—The reesterification of methyl laurate with glycerol was carried out as described above, but individual reaction parameters were varied to evaluate their effect on trilaurin formation. Separation procedures and analytical techniques remained unaltered.

Catalyst concentration. The reesterification was carried out varying the catalyst at 0.3, 0.35, 0.4, 0.45, and 0.5 per cent (based on weight of ester) using equivalent amount and 15-per cent excess methyl laurate to glycerol at 220°C under a nitrogen blanket. Samples were withdrwan at 1 hour intervals and analzed. Results are shown in Table 1.

TABLE 1.—*Trilaurin formation* under varying catalyst concentration at 220°C in a nitrogen atmosphere.*

Reaction Time hrs.	Sodium hydroxide concentration**, Per cent					
	0.3^{+}	0.35^{++}	0.4^{+}	0.4^{++}	0.45^{++}	0.50^{+}
3	28.3	33.9	48.3	61.6	59.0	41.9
4	30.0	42.3	52.9	61.6	63.8	44.5
5	38.2	46.3	58.0	64.8	64.0	46.9
6	43.0	49.7	56.8	71.7	68.1	57.6
7	44.6	52.2	-	69.9	67.1	60.2

* Expressed as per cent trilaurin
** Based on weight of methyl laurate
+ Equivalent amounts of methyl laurate and glycerol for reesterification
++ Fifteen-per cent excess methyl laurate used for reesterification.

Amount of excess methyl laurate. The per cent excess methyl laurate to glycerol was varied from equivalent amount to 30 per cent. The reaction was carried out at 220°C using 0.4-per cent NaOH catalyst under a nitrogen blanket. Samples were withdrawn every hour and analyzed. Results are shown in Table 2.

TABLE 2.—*Trilaurin formation* using varying amounts of excess methyl laurate at 220° with 0.4-per cent NaOH catalyst in a nitrogen atmosphere.*

Reaction Time hrs.	Excess methyl laurate, Per cent					
	0	10	15	20	25	30
3	48.3	54.4	61.6	60.7	44.8	52.8
4	52.9	56.6	61.6	63.6	58.8	53.7
5	58.0	54.8	64.8	66.0	59.3	59.5
6	56.8	61.4	71.7	63.2	55.8	54.1
7	-	-	69.9	-	62.3	-

* expressed as per cent trilaurin

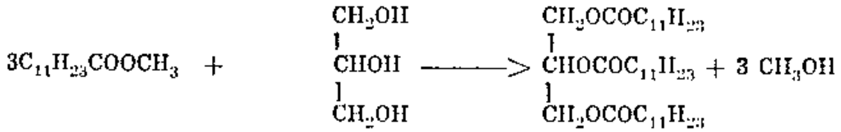
Reaction temperature. The reesterification was done at temperatures varying from 200° to 260°C using 15-per cent excess methyl laurate and 0.4-per cent NaOH catalyst under a nitrogen atmosphere. Samples were taken every hour and analyzed to give results shown in Figure 1.

Effect of pressure: For reactions done at 170°C and 200°C, respectively, the pressure was varied at 100 mm, 200 mm, and 760 mm. Results are shown in Figure 2.

Reproducibility of reesterification results. The reesterification of methyl laurate with glycerol was repeated several times using the most favorable conditions found for trilaurin formation to determine reproducibility of results.

RESULTS AND DISCUSSION

The alkali-catalyzed reaction is represented by the following equation:



$$3C_{11}H_{23}COOCH_3 \quad + \quad \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \longrightarrow \begin{array}{l} CH_2OCOC_{11}H_{23} \\ | \\ CHOCOC_{11}H_{23} \\ | \\ CH_2OCOC_{11}H_{23} \end{array} + 3\ CH_3OH$$

It is to be expected that partial reaction will produce monolaurin and dilaurin together with trilaurin and in a secondary reaction, soap formation is likely to occur between the partial products or FFA, if present, and the catalyst. These side reactions may retard the reesterification process through catalyst destruction (Choudhury, 1962).

In this investigation, the optimum conditions considered were those which produced the highest yield of trilaurin. Since trilaurin formation served as sufficient basis for the purpose of this study, the rest of the reaction products were not taken into consideration. Column chromatography with the aid of TLC was employed for its analysis. The procedure of Distler and Bauer was modified because methyl laurate interfered with trilaurin in the elution of the latter with benzene. This is to be expected since they have the same Rf value (0.41) as benzene due to the same functional group they possess. Hence it was necessary to go into a second step to separate them.

This was worked out by selecting the most suitable solvent from the Eluotropic Series of Solvents (Mangold, 1961). Carbon tetrachloride effected the clearest separation by TLC. (Rf values:

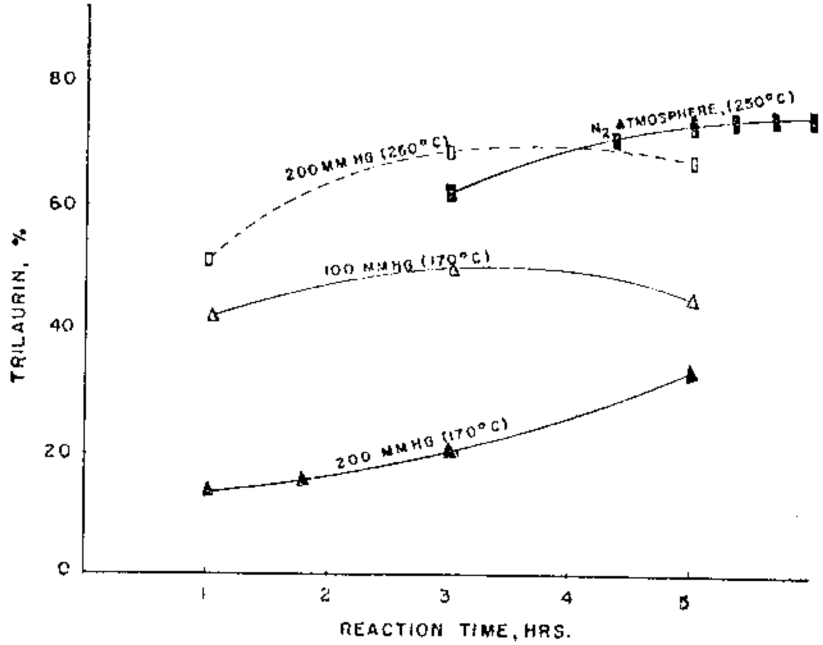


FIG. 1. Effect of reaction temperature on trilaurin formation using 15-per cent excess methyl laurate and 0.4-per cent NaOH catalyst under N_2 atmosphere.

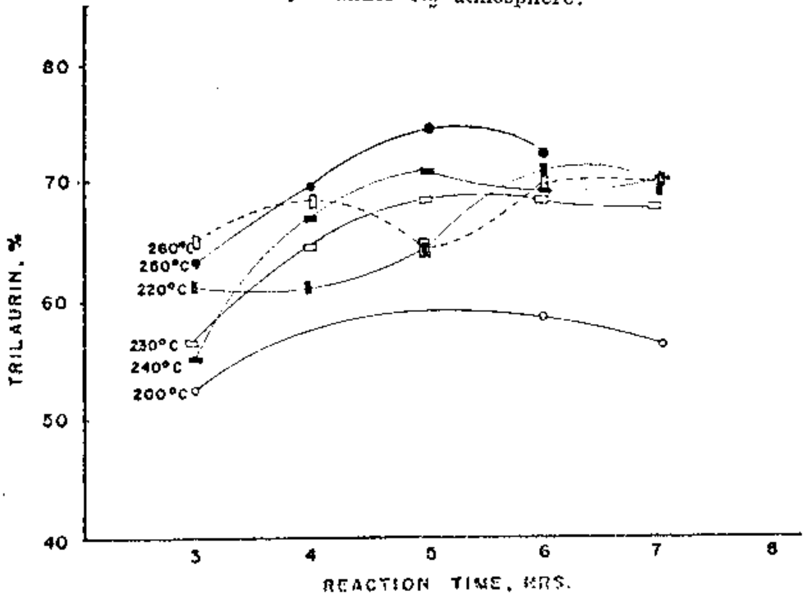


FIG. 2. Effect of pressure on trilaurin formation using 15-per cent excess methyl laurate in the presence of 0.4-per cent NaOH catalyst at specified temperature.

trilaurin, 0.0; methyl laurate, 0.58.) Methyl laurate was eluted first with carbon tetrachloride, followed by trilaurin which was eluted with benzene.

Before the effects caused by variations of reaction parameters could be assessed, it was necessary to determine the precision of the analytical procedure. The method was tested with authentic mixtures of trilaurin and methyl laurate. Results obtained varied within ± 0.8 per cent of the calculated values. The method was subsequently used in all determinations for trilaurin in this study. Aside from being simple, direct and rapid, the method gave fairly reproducible results:

a. Effect of catalyst concentration — Variations in the concentration of catalyst revealed that the reaction rate increased as the amount of catalyst is increased. Trilaurin formation is at its maximum at 0.4-per cent catalyst concentration (Table 1). The use of $ZnCl_2$ as catalyst caused the reaction mixture a certain degree of darkening. The reaction was rather violent.

b. Effect of reactant — As the amount of excess methyl laurate allowed to react with glycerol is increased, trilaurin formation correspondingly increased reaching its optimum with 15-per cent excess. (Table 2.) The reaction starts at about 160°C as indicated by the formation of methanol, a by-product. As the reaction proceeds, more and more glycerol is converted to monoglyceride which enter the ester phase, while the monoglyceride already in the ester phase continues to react to form di- and trilaurin. This behavior can be explained by the fact that glycerol is relatively insoluble in methyl laurate and that sodium hydroxide dissolves first in the glycerol phase. Probably as the excess of methyl laurate is increased, the solvent power of the ester for glycerol increases as the reaction proceeds and the maximum rate of reaction is attained just as the system becomes homogeneous. As the reaction nears completion, the rate correspondingly decreases (Gros and Feuge, 1949).

c. Effect of temperature — The curves in Figure 1 show that under the given conditions, an increase in temperature from 200° to 220°C resulted in an unusual increase in reaction rate. It is evident that reaction rate increases as the temperature is increased. Furthermore, above 220°C, a shorter time is required for the reaction mixture to attain equilibrium. Highest yield of trilaurin (74.2 per cent) was obtained at 250°C after 5 hours. The decrease in reaction rate observed at 260°C may probably be due to the formation of polyglycerols (Bailey, 1951) at such elevated temperatures. Also, at 260°C, the reaction mixture became highly colored even only after the third hour.

d. Effect of pressure — The effect of varying the pressure over the reacting mixture under the conditions specified are presented in Figure 2. The reaction mixture attained equilibrium faster at 100 than at 200-mm pressure. For experiments done at 170°C, the rate of reaction at 200-mm pressure is slower (33.5-per cent trilaurin after the 5th hour) compared to the results obtained at 100-mm pressure (51.6 per cent trilaurin after the third hour). A lower pressure then seems to favor the reaction. The experiment done at 250° and 200 mm, however, gave a remarkably high trilaurin conversion compared to that done at 170° although a marked darkening of the reaction mixture was observed. The reaction done at 250° under nitrogen at ordinary pressure gave the best result.

Unfortunately, pressures over the reacting mixture cannot be reduced below the boiling point of either free glycerol or methyl laurate. An experiment conducted at 0- to 1-mm pressure did not react because at 130°C, methyl laurate started distilling over.

Using the reaction conditions found most favorable for trilaurin formation, the average yield was 73.5 per cent with a standard deviation of ± 0.73 per cent. Purified trilaurin appears as needlelike crystals, m.p. 45 to 46°C (lit. value, 45.6°), saponification equivalent, 211.2.

SUMMARY

A simple, direct and fairly rapid method for the separation and determination of trilaurin in a reesterification mixture of methyl laurate and glycerol was developed using chromatographic techniques.

With the aid of this analytical method, a study of the various reaction parameters was undertaken and the conditions most favorable for trilaurin formation was established. Under these conditions, the average yield of trilaurin was 73.5 per cent with a standard deviation of ± 0.73 per cent.

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ALGINIC ACID, AGAR, AND CARRAGEENAN CONTENTS OF SOME PHILIPPINE MARINE ALGAE

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Ll. Arguelles, and Nerissa Sarabia
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TWO PLATES

ABSTRACT

Alginic acid, agar, and carrageenan, extractives from marine algæ, have important uses as emulsifiers, gelling and surfacing agents, stabilizers in food industries, pharmaceuticals, and other industrial processes. Samples of the brown (Phaeophyta) and of the red (Rhodophyta) algæ collected from exposed and protected marine waters of some Philippine provinces were analyzed for these extractives. Results are presented in Tables 1 and 2 of this study.

Marine algæ or seaweeds have been utilized by man for food and as fertilizers for centuries. In recent years, uses of their chemical extracts have rapidly expanded to constitute important components of food and pharmaceutical preparations and also in various industrial processings. This trend stimulated investigators to explore and exploit algæ as natural sources for the production of industrial extractives.

Seaweed utilization (Ash, 1954; Chapman, 1952; Duke University Marine Station, 1946; Johnston, 1968) has been the subject of study in foreign countries. Their use as food and as fertilizers dates back to early Roman times as reported in Chinese, Greek, and Roman writings. People from all parts of the world have been eating seaweeds for generations. In Japan, India, and the United States investigations have been made on the utilization of these aquatic plants as sources of alginic acid, agar, and carrageenan, extractives which have become ideal additives or vehicles in food and pharmaceutical manufacture. Rao and Mody (1968) worked on the extraction of alginic acid and alginates from brown seaweeds. Bashford *et al* (1950) reported on the manufacture of algal chemicals. Procedures for extraction of carrageenan were given in the works of Maton and Maton (1962), Stancroff (1965), Gordon and Jonas (1966), Smith and Montgomery (1959), Tressler and Lenon (1951), and Cerezo (1967). Most of them, however, were patented processes. Tseng (Nos. 350 and 351) gave detailed information on agar and algin, respectively, as to sources, chemical nature, properties and reactions, as well as uses and methods of commercial processes of manufacture.

Although Philippine seas abound with seaweeds of different species, only a few botanic surveys of some species have been undertaken. The latest was that by Velasquez *et al* (1971) involving collection and identification of the different species of algæ growing at different seasons of the year in the exposed and protected marine waters of Batangas and Bataan provinces. This study was supported by a grant from the National Research Council of the Philippines. Along side with this work the Biological Research Center of the National Institute of Science and Technology analyzed samples of species collected: of the brown algæ (Phaeophyta) for alginic acid and of the red algæ (Rhodophyta) for agar and carrageenan. The Philippines exports brown and red algæ to foreign countries, particularly to Japan and the United States, and imports the processed extractives needed for its growing food, pharmaceutical, textile, and rubber manufacturing industries. A chemical survey of locally available seaweeds may lead to the establishment of extraction plants for supply of domestic requirements and possibly for export of alginic acid, agar, and carrageenan.

Several studies on some chemical components of Philippine algæ have been published. De Leon *et al* (1963) determined the chemical composition of some Philippine algæ. Valenzuela *et al* (1951) investigated local seaweeds as sources of agar for pharmaceutical purposes. Manapat (1959) analyzed some Philippine brown algæ for alginic acid. Sulit and San Juan (1955) worked on the extraction of alginic acid from some species of Philippine sargassum.

Alginic acid, according to Kirk and Othmer (1954), is a seaweed constituent extracted from brown algæ where it occurs admixed with certain salts like sodium, ammonium, potassium, and calcium. As a crude extract, it is generally marketed as a white powder or in the form of a paste. Purified algin is marketed as pure sodium alginate, the sodium salt of alginic acid, or as a mixture of sodium alginate and other substances like dextrin, sucrose, and a phosphate. According to manufacturers, it is sold under different commercial names, among the most common ones are Dariloid, Dricoid; Kelcoloid, Kelgin, Keltex, and Superloid, all from Kelco Company of the United States, and Manucol, Collatex, and Manutex from Alginate Industries of England. The economic importance of alginic acid and its salts is based on its physical and chemical properties, according to Johnston (1968). Among its important uses are: as emulsifiers, as stabilizing, gelling and surfacing agents, and as synthetic films and fibers. It serves as emulsifiers and gelling agent in the cosmetic industry. As additives to food products; alginates are used in ice cream, sherbets, artificial creams, syrups, salad dressings, icings, cheese, and food concentrates. It prevents icing from adhering to cellophane wrappers because of its property to increase moisture retention. Films of alginates are utilized by meat processors in the preparation of sausage skins. Alginates are important stabilizing and thickening agents in the processing of natural and synthetic rubber latex. Absorbable surgical gauzes and threads are also prepared from alginates. Some of the important genera of brown algæ commonly known as commercial sources of alginic acid are *Macrocystis, Laminaria; Ascophyllum,* and *Fucus.*

Agar, another colloid extracted from species of the red algæ, comes in the market as powder, flakes, rectangular blocks, and in bundles of slender strips. It is also well known as agar-agar; the word being of Malayan origin, as reported by Tseng (No. 350). Many synonyms appearing in European markets include "Chinese moss," "seaweed isinglass," "Chinese isinglass," "Chinese gelatin." Agar readily dissolves in boiling water. Orientals have long relished agar as dessert in the form of sweetened or flavored gels. The use of agar as a solid bacterial medium was conceived by a German housewife, Frau Fanny Eilshemius Hesse who had been using agar in her kitchen as food. She passed the idea of using it as a bacteriological medium to her husband, a doctor, who in turn suggested it to Koch, his co-worker in research. It was then used in Koch's pioneering experiments on tubercle bacillus and published in 1882. This became a formal announcement of the use of agar as a new culture medium. Agar is now an indispensable material in microbiological laboratories. Agar has also established uses in pharmaceutical and cosmetic industries. *Gelidium* and *Gracilaria* spp. are the principal sources of agar.

Carrageenan is an algal carbohydrate with chemical structure and properties similar to agar (Smith and Montgomery;

1959). It is also extracted from red algæ but dissolves in cold water. Like alginates, it is used to stabilize emulsions and suspend solids in the food, textile, and pharmaceutical industries.

MATERIALS AND METHODS

Collection and preparation of algæ.—Samples of algal species belonging to the brown (for alginic acid) and red (for agar and carrageenan) algæ were supplied and identified by Velasquez *et al* (1971) from their collections at different seasons of the year, mostly in the exposed and protected marine waters of Batangas and Bataan provinces, site of their first year of study. Since their project lasted for 3 years, collection sites were extended to other Philippine marine waters. The dried samples were kept in dry, clean glass jars, properly labeled preparatory to chemical analyses.

Moisture determination.—The moisture content (AOAC, 1970) was determined by drying to constant weight, 1-gram powdered sample in an electric Precision oven at 100°C.

Determination of alginic acid.—The procedure of Cameron *et al* (1948) for the extraction of alginic acid was followed although some modifications were introduced. A sample (0.5 g) of dried seaweed, ground to pass a 65-mesh sieve was treated with 10 ml of 0.2N sulfuric acid and allowed to stand overnight. The acidified sample was filtered. The residue was washed with water, then transferred to a breaker with 20 ml distilled water. An equal volume of 6-per cent sodium carbonate was added and the mixture kept at 50°C for 2 hours, with occasional stirring and then left overnight. After filtration, the filter was washed with 20 ml of 1.5-per cent sodium carbonate solution followed by distilled water. Filtrate and washings were transferred to a tap funnel with a fine exit tube and run slowly through a fine jet into 30 ml of 10-per cent calcium chloride solution, stirring gently. The resulting precipitate was allowed to stand for at least 2 hours. The precipitate was filtered and N hydrochloric acid was added slowly until all the carbonates have been driven off. The alginic acid precipitate was washed, dried at 80°C, and weighed. Results were expressed as percentage of alginic acid based on moisture-free sample.

Extraction of agar.—Among the analytical procedures tried for extraction of agar, the method of de Leon *et al* (1947) was selected. To 100 grams of the bleached dry weed, 500-ml tap water heated to 60°C and containing 2.5-g commercial sodium thiosulfate was poured and the algæ permitted to soak for 2 hours, while the temperature was allowed to fall for the rest of the time. The soaked material was then washed with water and allowed to drain. After draining, it was transferred to a breaker containing 3.5 liters of boiling water acidified with 0.25 ml of concentrated sulfuric acid stirring constantly for about 30 minutes to 1 hour to hasten the extraction. The mixture was strained through cheesecloth and permitted to congeal. The solidified samples were then subjected to freezing temperature for about 8 to 10 hours and then transferred to suitable containers and exposed to the sun to hasten the thawing process. The samples were dried in a vacuum oven at 80°C and weighed. Percentage agar was calculated based on moisture-free sample.

Extraction of carrageenan.—Based on the procedure of Cerezo (1967), 60 g of dried algæ, cut into small pieces was extracted with 3 liters of water at room temperature for 24 hours. Continuous mechanical stirring was done during the extraction. The viscous suspension obtained was decanted, centrifuged, and the supernatant liquid poured into 4 volumes of ethanol. The crude carrageenan precipitated as long fibers. Extraction was repeated until no further precipitate was obtained upon pouring the extract into 4 times its volume of ethanol. The precipitate was left overnight in alcohol, filtered and dried at 60°C in a Precision oven. Carrageenan content was expressed as percentage based in the moisture-free sample.

RESULTS AND DISCUSSION

Thirty samples consisting of 27 species belonging to the brown algæ were analyzed for alginic acid content. Table 1 shows that *Turbinaria ornata* (Plate 2, fig. 1) taken from Puerto Galera, Mindoro, a protected shore, gave the highest yield of alginic acid, 46.76 per cent, followed by *Hormophysa triquetra* from Alabat, Quezon, which yielded 41.81 per cent. The lowest yield, 7.10 per cent, was obtained from a species of *Hydroclathrus* collected from Moron, Bataan.

It is known that ecology and seasonal variations could influence the alginic acid yields of algæ. From Table 1, however, no safe conclusions can be drawn regarding the influence of such factors, since the samples analyzed depended on those supplied by the collection team. Most of the algæ were identified only ac-

TABLE 1.—*Alginic acid content of some Philippine brown algæ.*

Collection		Description of collection site	Scientific name	Alginic acid (moisture-free basis)
Date	Place			
				Per cent
October 1969	Calatagan, Batangas (lighthouse)	exposed	*Padina japonica*	11.83
January 1970	Orion, Bataan	protected	*Padina* sp.	24.12
May 1970	Moron, Bataan	exposed	do	10.15
October 1971	Alabat, Quezon	do	do	19.63
October 1969	Punong Pandil, Nagbalayan, Moron, Bataan	do	do	24.08
Do	Sitio, Emon, Moron, Bataan	do	*Sargassum* sp.	28.00
Do	Bo. Panibatuhan Moron, Bataan	do	do	31.34
Do	Bo. Sabang, Moron, Bataan	do	do	25.24
July 1970	Orion, Bataan	protected	do	24.33
October 1970	Santiago Island Pangasinan	exposed	do	25.60
May 1971	Moron, Bataan	do	do	24.17
October 1971	Alabat, Quezon	protected	do	32.31
March 1971	Hundred Islands Pangasinan	exposed	do	11.83
March 1970	do	do	*Hydroclathrus clathratus*	7.28
Do	do	do	do	18.14
April 1971	Boguete, Puerto Galera, Or. Mindoro	protected	do	22.07
May 1970	Moron, Bataan	exposed	*Hydroclathrus* sp.	20.40
Do	do	do	do	7.10
March 1970	Hundred Islands Pangasinan	do	*Colpomenia sinuosa*	12.17
May 1971	Medio Island, Puerto Galera, Or. Mindoro	do	do	23.27
May 1970	Moron, Bataan	do	*Colpomenia* sp.	10.23
March 1970	Hundred Islands Pangasinan	do	*Dictyota linearis*	14.26
May 1970	Moron, Bataan	do	*Dictyota* sp.	14.23
Do	do	do	do	13.37
October 1971	Alabat, Quezon	protected	do	25.12
Do	do	do	do	29.38
May 1970	Butong, Batangas	do	*Chnoospora* sp.	21.10
October 1970	Santiago Island Pangasinan	exposed	Unidentified sp	23.80
May 1971	Recodo cave, Puerto Galera, Or. Mindoro	protected	*Turbinaria ornata*	46.76
October 1971	Alabat, Quezon	do	*Hormophysa triquetra*	41.81

Figures are averages of triplicate determinations.

cording to genera and the species were not known, aside from the different places of collection. In the case of *Hydroclathrus clathratus,* two samples of the same species collected at the same time and same place, Hundred Islands, Pangasinan, one contained 7.28-per cent alginic acid, while the other gave 18.14 per cent. Results of the analyses showed that from the nine species of *Sargassum,* only one from Hundred Islands, Pangasinan, gave a low yield of 11.83 per cent, the other eight, collected from various places of Bataan, Quezon, and Pangasinan, ranged from 24.17 to 32.31 per cent.

The extraction of alginates is based on the principle that alginic acid is insoluble in water but can easily be converted into the water-soluble sodium salt. Washings after calcium chloride treatment eliminates laminarin, mannitol, and other salts present in brown algæ, and alginic acid subsequently precipitated by strong mineral acid.

Twenty-nine samples of the red algæ representing 25 species were each analyzed separately for agar and carrageenan contents; results are given in Table 2. Agar and carrageenan are both present in the red algæ. The extraction of agar from the seaweed is based on the principle that it is soluble in hot water but relatively insoluble in cold water.

According to Humm (1948), in his study of two Brazilian seaweeds; *Hypnea musciformis* var. *hipporoides* should contain more than 35 per cent of an excellent quality agar. Ten of the samples representing nine species gave high yields of agar, varying from 34.20 (*Eucheuma* sp.) to 58.15 per cent (*Gracilaria eucheumoides*, Plate 1, fig. 2). People in coastal towns are familiar with *Gracilaria* spp. since these are the species they use in preparing sweetened desserts. Of the seven samples of *Gracilaria* represented by five species that were analyzed, two samples showed no gelling of the extracts, indicating low yields of agar, while the other five samples gave 38.23 to 58.15 per cent.

All the red algæ contained carrageenan ranging from 1.97 (*Asparagopsis* sp.) to 54.25 per cent (*Eucheuma* sp., Plate 2, fig. 2). While *Eucheuma* spp. are well known to be good sources of carrageenan, out of the nine species analyzed only four yielded more than 25 per cent. Table 2 reveals that *Halymenia* spp., in Plate 1, fig. 1, are also good sources of carrageenan. Only four species of *Halymenia* were analyzed and the yields were all high, ranging from 40.33 to 51.30 per cent. It is interesting to note

TABLE 2.—*Agar and carrageenan contents of some Philippine algæ.*

Collection		Description of collection site	Scientific name	Agar	Carrageenan
Date	Place			(Moisture-free basis)	
				Per cent	
October 1969	Punong Pandil Nagbalayon, Moron, Bataan	exposed	*Halymenia durvilleai* Bory	No gel formation	40.33
January 1970	Butong beach, San Luis, Batangas	protected	do	do	51.40
October 1970	Pangasinan	exposed	*Halymenia* sp.	do	42.92
May 1970	Moron, Bataan	do	do	do	41.91
October 1969	Calatagan, (lighthouse) Batangas	do	*Chondrococcus hornemanii*	do	20.84
Do	do	do	*Gracilaria verrucosa*	48.21	7.24
February 1970	Calatagan, Batangas	do	do	43.90	3.53
May 1970	Moron, Bataan	do	*Gracilaria* sp.	No gel formation	6.46
Do	Butong, Batangas	protected	do	38.23	11.73
Do	do	do	*Gracilaria eucheumoides*	No gel formation	10.09
October 1970	Santiago Island, Panatagan,	do	do	58.15	7.63
December 1970	Do	do	*Gracilaria* sp.	43.40	10.73
January 1970	Butong beach, San Luis, Batangas	do	*Eucheuma* sp.	35.0	7.11
May 1970	Moron, Bataan	exposed	do	34.20	4.82
July 1970	Antique	do	do	No gel formation	54.25
Do	Bolinao, Pangasinan	do	*Eucheuma cottonii* (crawling type)	do	35.86
July 1970	Cebu	do	*Eucheuma cottonii* (erect type)	do	25.97
October 1970	Pangasinan	protected	*Eucheuma cottonii*	do	5.72
December 1970	Santiago Island, Pangasinan	do	*Eucheuma* (prostrate) *cottonii*	52.43	18.77
May 1971	do	do	*Eucheuma cottonii*	No gel formation	26.42
February 1970	Calatagan, Batangas	exposed	*Laurencia papilosa*	48.50	6.37
May 1970	Moron, Bataan	do	*Laurencia* sp.	No gel formation	9.32
March 1971	Santiago Island Pangasinan	protected	do	4.68	10.09
October 1971	do	do	do	15.40	6.34
May 1971	San Isidro, Puerto Galera, Or. Mindoro	exposed	*Asparagopsis* sp.	No gel formation	1.97
October 1971	Panhabulan, Alabat, Quezon	protected	*Acanthophora spicifera*	29.47	14.10
do	Alabat, Quezon	do	*Gelidiopsis* sp.	No gel formation	3.02
February 1970	Calatagan, Batangas	exposed	*Gelidiela acerosa*	do	2.94

Figures are averages of triplicate determinations.

that *Eucheuma cottonii* (prostrate) yielded 52.43-per cent agar and also 18.77-per cent carrageenan.

Table 2 also shows that five species contained insignificant quantities of both agar and carrageenan.

SUMMARY

Samples of different species of marine seaweeds belonging to the brown algæ (Phaeophyta) and to the red algæ (Rhodophyta) collected at different seasons of the year from exposed and protected marine waters were analyzed for industrial extractives — alginic acid, agar, and carrageenan.

Thirty samples consisting of 27 species of the brown algæ were analyzed for alginic acid. *Turbinaria ornata* J. Agardh, taken from Puerto Galera, Mindoro, gave the highest yield of 46.76 per cent; *Hydroclathrus* sp. gave the lowest, 7.10 per cent.

Twenty nine samples of red algæ representing 25 species were each analyzed separately for agar and carrageenan. Ten of the samples representing nine species gave high yields of agar, varying from 34.20 *(Eucheuma* sp.) to 58.15 per cent *(Gracilaria eucheumoides* Harvey).

All the 29 samples of red algæ contained carrageenan ranging from 1.97 *(Asparagopsis* sp.) to 54.25 per cent *(Eucheuma* sp.). While *Eucheuma* spp. are known to be good sources of carrageenan. *Halymenia* spp. were also found to yield from 40.33 to 51.30 per cent of the said hydrocolloid.

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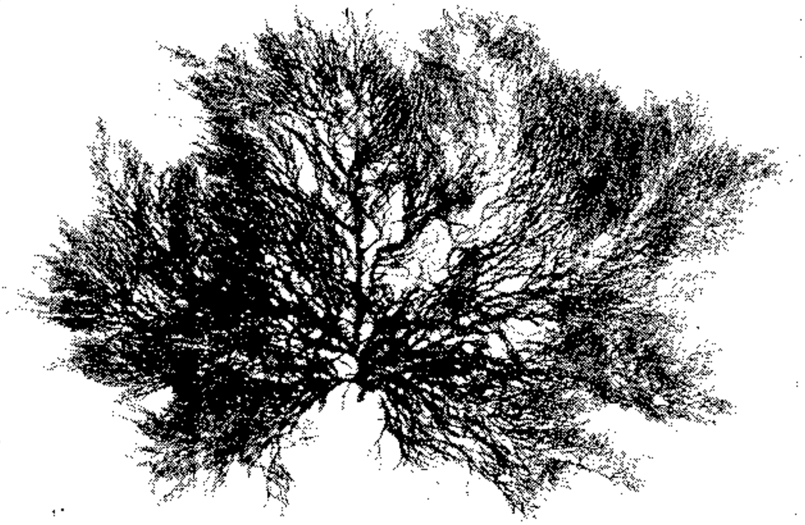
ILLUSTRATIONS

PLATE 1

FIG. 1. *Halymenia durvilleai* Bory × 1/3, species containing carrageenan.
2. *Gracilaria eucheumoides* Harvey × 1/2, a good source of agar.

PLATE 2

FIG. 1. *Turbinaria ornata* J. Agardh × 2/3, source of alginic acid.
2. *Eucheuma* sp. × 1/2, a carrageenan-yielding species of red algæ.



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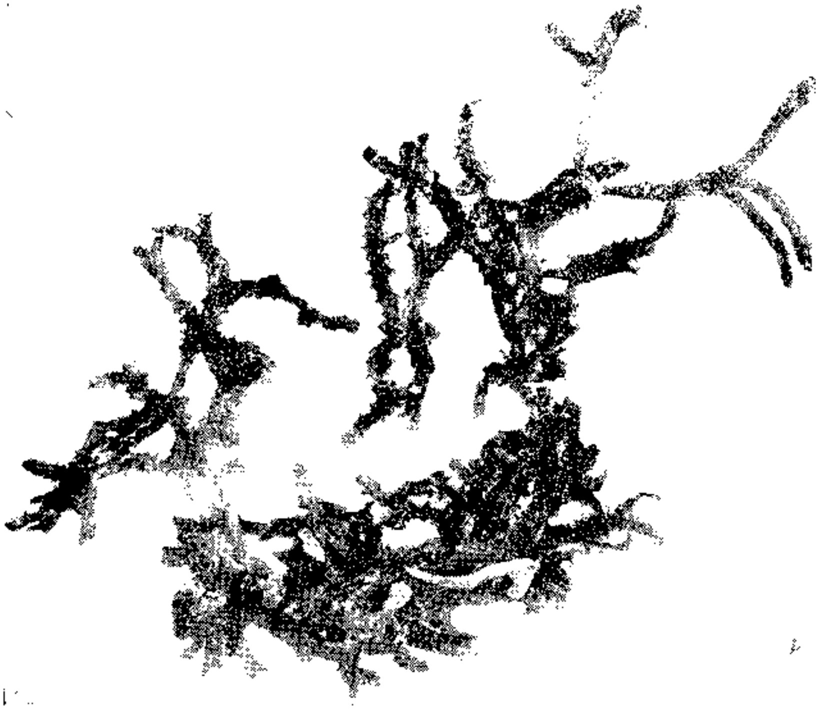

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PLATE 1

1

2

PLATE 2

GROWTH CHARACTERISTICS AND THERMAL RESISTANCE OF SPOILAGE ORGANISMS ISOLATED FROM CANNED PEACHY PAPAYA GIVEN MINIMAL HEAT TREATMENT

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ONE TEXT FIGURE

ABSTRACT

The morphological and physiological characteristics of the spoilage microorganisms isolated from canned peachy papaya given minimal heat treatment were studied. Of the three isolates obtained, two were gram-positive rods, nonsporeforming, motile and catalase-negative. These isolates did not liquefy gelatin and were microaerophilic with respect to oxygen requirement. On prolonged incubation (2 weeks) at 37°C, both were able to develop cream pigment in orange serum agar. These two isolates differed only in their ability to ferment arabinose. The third isolate was gram-positive, motile coccus, which was nonsporeforming, microaerophilic, did not liquefy gelatin and reacted positively towards the catalase test.

A representative result of the thermal resistance studies for one of the isolates is presented. It was generally observed that the D values of this isolate in phosphate buffer were higher than in peachy papaya.

INTRODUCTION

Thermal processing is a method of preserving food by the use of high temperature to destroy living organisms capable of causing deterioration of the food and endangering the health of the consumer. The heat cooks the food and changes its flavor and physical characteristics. It destroys some or all of the microorganisms and renders inactive any that remains. It also destroys the activity of the enzyme.

For a proper understanding of the problems which arise in the heat processing of canned foods: it is essential to know the principles involved and the various factors which influence the heat destruction of microorganisms. In the development of adequate heat processes for canned foods, consideration must be given to the heat resistance of the significant spoilage organisms. The relatively lengthy heat treatment necessary to destroy spoilage organisms often results in the deterioration of organoleptic quality of the food. The appearance, flavor and nutritive value may also be impaired to some degree. Fruits and vegetables are generally known to be sensitive to heat, hence it is important to know the adequate time-temperature relationship of processing. Processing requirements are met when a compromise between retaining the organoleptic quality and preserving the food for a reasonable length of time is achieved.

The thermal resistance of a microorganism is determined by measuring its D and Z values. Stumbo (1965) defined the D value as the time required at any temperature to destroy 90 per cent of the spores or vegetative cells of a given organism. It is numerically equal to the number of minutes required for the survivor curve to traverse one log cycle. It is mathematically equal to the reciprocal of the slope of the survivor curve. The Z value is the number of degrees in Fahrenheit required for the thermal destruction curve to traverse one log cycle. It is mathematically equal to the reciprocal of the slope of the thermal destruction curve. It is used in process calculations to account for the relative resistance of a microorganism to different temperatures in a particular medium.

It is very well known that heat resistance of a particular organism depends on many factors. Stumbo (1965) and Hersom and Hulland (1969) enumerated the following factors: inherent resistance, age, environmental influence active during the growth and formation of cells or spores, growth temperature, nature of medium in which spores are produced, influences active during the time of heating of the cells or spores, nature of the medium in which bacteria are suspended when heated, the pH of the medium, concentration of sodium chloride; sugars, other carbohydrates, fats, curing agents, water content, antibiotics and spices.

The objective of this study is to isolate the significant spoilage organisms in canned peachy papaya and to study their growth and thermal resistance characteristics in order to have a basis for the establishment of an adequate heat process for this product.

EXPERIMENTAL PROCEDURE

Preparation of canned peachy papaya.—Peachy papaya is a canned product consisting of ½" cubes of orange-yellow colored, medium-ripe papaya in 35°Brix syrup with the addition of 0.6-

per cent citric acid and 0.02-per cent almond extract for flavoring, free from seeds, peels or any other extraneous material.

Peachy papaya was prepared following a standard procedure of UP Pilot Food Plant (UP-PFP) but varying the processing conditions to produce underprocessed batches. Control samples were simultaneously prepared. Medium-ripe pieces of papaya which were well trimmed, fairly well formed, smooth, free from decay, breakdown and internal hard lumps were used in the preparation. After determining the weight of the fruits as purchased (AP), they were soaked in chlorinated water (1 tbsp of Milton antiseptic solution per liter of water) for 15 minutes and then rinsed well in tap water. Stem ends, seeds and soft tissues were removed after peeling. Using a meat slicer, the fruit halves were sliced into ½" thick slices and finally cut into ½" cubes manually. The cubes were washed in tap water and the edible-portion weight determined. Cans were filled with the cubes (300 g per # 22 can) and the prepared 35°B syrup with 0.6-per cent citric acid and 0.02-per cent almond extract was added to 1/8" headspace. The cans were exhausted at 180°F for 5 min.; processed at 212°F for 10 min. and then cooled (control). Variations of this general procedure were made according to the need of the experiment. The variations made were the following:

A. After filling, the cans were sealed, inverted and cooled.

B. The filled cans were exhausted in water bath at 82.2°C (180°F) for 5 minutes, sealed, inverted and cooled.

C. The filled cans were exhausted in a water bath at 82.2°C (180°F) for 5 minutes, sealed, processed at 100°C (212°F) for 2 minutes and cooled.

D. The filled cans were exhausted in water bath at 82.2°C (180°F) for 5 min., sealed, processed for 4 min. at 100°C (212°F) and then cooled.

Sample cans were divided into three sets, each set containing the 4 variations plus one control. The first set was stored at room temperature, the second set was at 37°C and the third set at 55°C. The cans were observed daily for visible signs of micspoilage during storage for various lengths of time prior to microbial analysis. Examination of cans with seemingly normal appearance was performed after 1 month storage. Examination of bulging cans of the commercially available peachy papaya, said to have been processed for 10 minutes in boiling water bath after exhausting at 180°F for 5 minutes, was also performed.

Isolation of microorganisms.—The sample cans were coded and their external conditions were noted and recorded. One end including an inch of the can wall was cleaned with 95-per cent ethyl alcohol and then covered with a sterile petri dish. The sterile end was punctured with a sterile steel probe and gas samples were collected by water displacement. Due to the great pressure of the gas in the can, gas collection was not possible. After noting the appearance of the product, the contents of the can were homogenized in a sterile blendor. Internal appearance of the cans were noted after emptying the contents.

A 20-gram sample was aseptically withdrawn and placed into a sterile stoppered tube. For each sample can, four tubes of glucose tryptone broth and four tubes of freshly exhausted (20 minutes in boiling water bath) liver broth with crushed tomatoes (National Canners Association, 1968) were used for culturing. One-half of the set of tubes of each medium were each inoculated with 3 to 4 ml of homogenized samples (large sample) and the other half with 0.3 to 0.4 ml of the homogenized sample (small sample). All tubes with liver broth with crushed tomatoes were stratified with sterile vaspar (1 part paraffin, 1 part vaseline, and 4 parts mineral oil). Half of the tubes of each of the inculated medium (two containing the large and two containing the small) were incubated at 37°C (98.6°F) while the other half was incubated at 55°C (131°F).

Smears from the original sample were prepared and stained for microscopic examination. The pH of the sample was then determined. Examination of culture was made every other day. Isolation was performed at least once a week by streaking into orange serum agar plates and nutrient agar plates. Those that appeared to be typical bacterial colonies and isolated from among the colonies on the plates were chosen. Isolated colonies obtained were examined microscopically and finally streaked into orange serum agar slants and nutrient agar slant.

CHARACTERIZATION

The morphological and physiological characteristics of the isolates were studied. Due to limited laboratory facilities only their reaction towards the catalase test, their ability to liquefy gelatin, their oxygen requirements, and their ability to ferment various sugar and sugar alcohols were determined.

Thermal resistance studies.—A modification of the unsealed thermal death time (TDT) method by Schmidt (1950) was used. Test tubes (pyrex) used in this experiment were 100.5 mm long with an inside diameter of 11.15 mm and a thickness of 0.8 mm.

Preparation of media for TDT determination.—Phosphate buffer and homogenized peachy papaya were the heating media used while orange serum broth was the subculture medium. Phosphate buffer medium, pH 7, was prepared by mixing M/15 Na_2HPO_4 and M/15 KH_2PO_4. The buffer was autoclaved in flasks for 20 minutes at 121°C. The peachy papaya medium was prepared by homogenizing the contents of unspoiled cans of the product processed at 100°C for 10 minutes.

Preparation of TDT tubes.—Prepared media were dispensed aseptically into sterile TDT tubes. The peachy papaya TDT tubes were heated for 15 minutes in a boiling water bath. After heating, the tubes were placed inside the drying oven at 250°F for a few hours to dry the moistened cotton plugs.

Preparation of cell suspension.—A 72-hour culture of the PP_5 isolate in orange serum agar slant was used. The cells were collected by scraping the cells on the surface of the agar slant and then suspending them in sterile distilled water. The approximate number of cells in the original cell suspension was determined by direct microscopic count using the Neubauer cell counter observed under a Spencer phase — contrast microscope. Viable cells were determined by making total plate count at 37°C using orange serum agar.

Time-temperature measurements.—Desired dilutions of the above cell suspension were made such that each milliliter contained approximately 10^5 to 10^6 cells. Each TDT tube was inoculated with 0.1 ml of the cell suspension. Six inoculated tubes and one uninoculated control tube for every time-temperature combination were used. A thermostatically controlled water bath with shaker was used for heating. The temperatures used were 150°F, 160°F, 170°F, 180°F, and 190°F for the phosphate buffer tubes and 135°F, 150°F, 160°F, 170°F, and 180°F for the peachy papaya tubes. The heating time was varied from 1 to 15 minutes depending on the temperature used. For every specified temperature, three sets of TDT tubes (six inoculated and one control tube per set) were placed in a steel test tube rack and then heated in the water bath till the specified time was finished. The tubes were immediately cooled in cold water bath. Each tube was then labelled with the appropriate variable.

Measured amounts of orange serum broth were poured into each of the phosphate buffer TDT tubes. They were then incubated for at least 72 hours at 37°C before observation. Positive tubes of peachy papaya could not be observed due to the viscosity and turbidity of the medium, therefore subculturing after 72-hour incubation was performed. A loopful of the culture in peachy papaya was inoculated into orange serum broth. These inoculated orange serum broth tubes were then incubated for 72 hours at 37°C. Positive tubes, indicated by turbidity were observed and recorded.

Calculations.—D values were calculated using formula of Halvorson and Ziegler (1932): $D = \frac{U}{\log a - \log b}$, where U = time of heating at a given temperature, a = initial number of cells present in all replicate samples, b = most probable number of cells surviving each time-temperature relationship in all replicate samples.

Thermal destruction (TD) curves were constructed using the calculated D values. In the construction of thermal destruction curve, average D values were taken in cases where there were more than one. This end point technique was used by Stumbo (1965) to obtain TD curves. Many workers have found this technique to be reliable for obtaining parameters to count for bacterial resistance in calculation of sterilizing process (Stumbo, 1965).

RESULTS AND DISCUSSIONS

It was observed that not all underprocessed cans became spoiled after a storage period of 1 month to 1 year. It might be that the amount of contaminants was too small to cause spoilage. Cheftel and Thomas (1965) stated that a heat treatment applied to a given product can be sufficient or insufficient, depending on the number of microorganisms initially present.

Examination of normally appearing cans which previously had received minimal heat treatment gave negative results microbiologically after 1 month storage. Examination of bulging cans from the commercially available peachy papaya, which previously had been processed at the recommended processing technique, gave negative results microbiologically. It was believed that the spoilage was chemical rather than bacterial. (National Canners Association, 1968.)

Three isolates were obtained from several cans examined. Of the three isolates, two were rod-shaped and the other was cocci-shaped. One rod-shaped isolate (PP_5 isolate) was taken from a spoiled can (labelled PP_5) with a mixed flora of bacteria and yeasts. The other two isolates (PP_7-1 and -2) were both taken from a spoiled can (labelled PP_7) with mixed cultures of rods, cocci and yeasts.

These two cans, PP_5 and PP_7, were under-processed. Both were exhausted at 82°C (180°F) for 5 minutes, sealed and not processed. PP_5 was stored for 15 days and PP_7 was stored for 10 months at ordinary room temperature prior to examination. The cans when examined were bulging and were of the hard swell type. The product in both cases appeared mashy and its syrup was frothy and cloudy. No corrosion was observed in both cans. The spoiled product of PP_5 had a pH of 3.70 while that of PP_7 had a pH of 3.60.

Growth characteristics of the three isolates are tabulated in Table 1.

It was found that PP_5 and PP_7-1 isolates were nonspore forming, gram-positive rods, microaerophilic and motile. Both developed cream pigment with sheen (luster) on prolonged incubation at 37°C on orange serum agar. Both isolates were catalase-negative and did not liquefy gelatin. Test for sugar fermentation showed that both were able to ferment galactose, mannose, xylose, and dextrose but were not able to ferment mannitol, dulcitol, lactose, rhamnose, and inositol. The PP_5 isolate was able to ferment arabinose while the PP_7-1 isolate did not produce acid from this sugar. No gas was produced in any of the Durham tubes.

PP_7-2 isolate was a gram-positive cocci. It was a nonspore former, microaerophilic, catalase positive, motile and did not liquefy gelatin. On prolonged incubation at 37°C, this isolate did not develop any particular pigment. Growth on surface of agar was thick and slimy. Sugars fermented were mannitol, mannose, lactose, and dextrose. Acids were produced, but no gas was evident in the Durham tubes.

A representative result of the thermal resistance determination of the PP_5 isolate is tabulated in Table 2. Average D values are plotted in Figure 1.

TABLE 1.—*Growth characteristics of peachy papaya isolates.*

Growth characteristics	PP$_5$ isolate	PP$_7$-1- isolate	PP$_7$-2- isolate
Shape	rod	rod	cocci
Gram stain reaction	Gram +	Gram +	Gram +
Growth on surface of agar	scanty	scanty	thick, slimy
Oxygen requirement (as observed during plating	micro-aerophilic	micro-aerophilic	micro-aerophilic
Optimum temp. for growth	37°C	37°C	37°C
Spore formation	nonspore former	nonspore former	nonspore former
Pigment development on prolonged incubation at 37°C on orange serum agar.	creamy with sheen	creamy with sheen	translucent white
Test for motility			
a. Hanging drop	motile	motile	motile
b. Motility agar stab	scattered growth	scattered growth	scattered growth
Gelatin liquefaction	-	-	-
Catalase reaction by H_2O_2	-	-	+, bubbles
Test for sugar, 1 per cent in purple broth base (acid reaction)			
a. mannitol	-	-	+
b. arabinose	+	-	-
c. galactose	+	+	-
d. dulcitol	-	-	-
e. mannose	+	+	+
f. xylose	+	+	-
g. lactose	-	-	+
h. dextrose	+	+	+
i. rhamnose	-	-	-
j. inositol	-	-	-

The TD curve obtained is shown in Fig. 1. It was observed that D values of the microorganisms in phosphate buffer were higher than in peachy papaya. This may be due to a difference in pH values; phosphate buffer had a pH 7 while peachy papaya had a pH 3.9. This finding is in conformity with the reports given by Sognefest *et al* (1948) that the resistance of a microorganism is maximum when the medium is approximately neutral and rapidly decreases as the hydrogen ion-concentration approaches both acid and alkaline ranges. Experiments by Baumgartner and Knocks (1940) showed that nonsporeforming bacteria gave maximum heat resistance at pH value corresponding to maximum hydration of the bacterial protein; as the organism would in such case be less susceptible to harmful agents. This pH varies with different types of microorganisms.

This preliminary finding shows the importance of determining the thermal resistance characteristics of a spoilage organism

TABLE 2.—*Thermal resistance characteristics of PP_5 isolate.*

a. Thermal resistance using phosphate buffer pH 7

Temp.	Time (min)	No. of: tubes Positive	Negative	b*	D**	Ave. D
150°F	5	6	0			
	10	6	0			
	15	5	1	10.73	2.55	2.55
160	4	6	0			
	7	6	0			
	9	5	1	10.73	1.53	1.53
170	2	6	0			
	3	5	1	10.73	0.51	
	6	4	2	6.58	0.98	0.74
180	1.5	6	0			
	2	5	1	10.73	0.34	
	5	1	5	1.09	0.73	0.53
190	1.5	1	5	1.09	0.21	
	2	2	4	2.43	0.30	0.25
	4	0	6	–		

b. Thermal resistance using homogenized peachy papaya

Temp.	Time (min)	No. of tubes Positive	Negative	b*	D**	Ave. D
135	3	6	0			
	4	5	1	10.73	0.68	
	5	1	5	1.09	0.72	0.70
150	1	6	0			
	2	5	1	10.73	0.34	
	3	4	2	6.58	0.49	0.42
160	1	6	0			
	2	2	4	2.43	0.30	0.30
	3	0	6			
170	1.5	0	6			
	2	1	5	1.09	0.29	0.29
	3	0	6			
180	1.5	0	6			
	2	0	6			
	3	0	6			

a—8.04 x 10^6 cells — The initial number of cells present in all replicate samples.

* Most probable number of cells surviving each time-temperature relationship

** $\frac{U}{\log a - \log b}$, U = time of heating

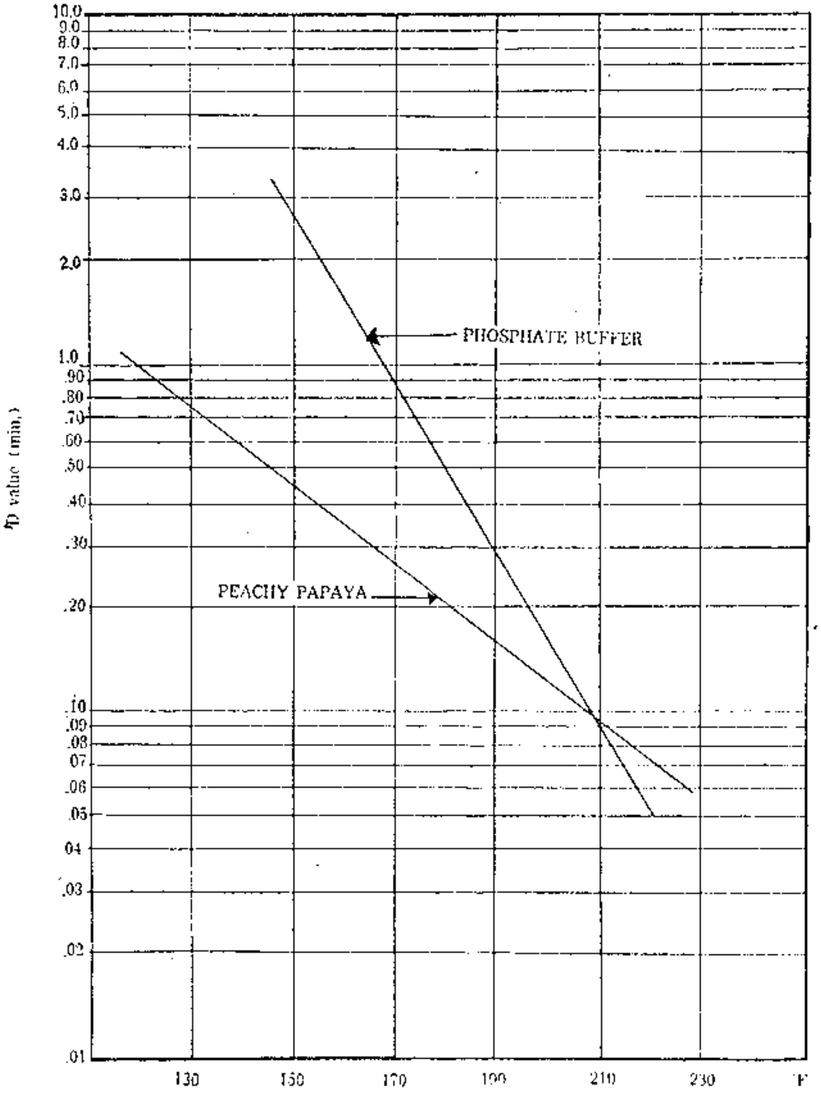

FIG. 1. Thermal destruction curves of PP^5 isolate using phosphate buffer and homogenized papaya as the heating media.

isolated from the food product with respect to the natural food medium itself as a basis for determining the adequate heat treatment needed for processing a specific type of food product.

SUMMARY AND CONCLUSION

Canned peachy papaya given minimal heat treatment and the recommended processing technique were prepared. These cans were stored for 1 month and/or until signs of spoilage were noted. Three isolates were obtained after several cans were examined microbiologically. Growth characteristics of these isolates were studied. The thermal resistance of one of the isolates was determined. The D values in phosphate buffer were found to be greater than those in peachy papaya. However, the significance of these PP_3 isolates in the determination of the most heat-resistant spoilage microorganism in peachy papaya cannot yet be established. More work on the isolation and thermal resistance have yet to be undertaken.

While a significant beginning in this area has been made, a great deal of work still remains to be done in the microbial phase of the standardization of thermal processes of local canned food products.

ACKNOWLEDGMENT

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SOGNEFEST, P., G. HAYS, E. WHEATON, and H. BENJAMIN (1948). Effect of pH on thermal process requirements of canned foods. Food Res. **13**: 400-416.

STUMBO, C. R. (1965). Thermobacteriology in Food Processing. New York: Academic Press, 236 pp.

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ELECTRONIC AND STRUCTURAL EFFECTS ON RATES AND EQUILIBRIA, VI

POLAROGRAPHIC REDUCTION OF SUBSTITUTED BENZALDEHYDES*

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and
WENDEL Y. LIM
Philippine Refining Company, Manila

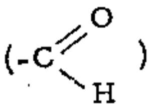
TEN TEXT FIGURES

ABSTRACT

Polarographic half-wave potentials, $E_{\frac{1}{2}}$, of 14 different benzaldehydes were measured under identical conditions. A linear free energy correlation of this polarographic reduction showed that σ n correlates better than σ as the set of substituent constants and that *ortho*-effect operates in the 2-substituted benzaldehydes. Results are explained in the light of modern electronic and structural concepts.

In our attempt to investigate whether chemical heterogeneous systems follow the same electronic and structural effects exhibited by homogeneous processes, we embarked on the study of the polarographic reduction of nitroarenes [Ku and Lim (1971)]. In our polarographic study of nitroarenes, we observed that our model compounds showed a good linear free energy relationship and was found to follow electronic and structural trends given in the literature [Kolthoff and Lingane (1962), Zuman and Kolthoff (1962), Zuman (1967 and 1969)].

As an extension of this interesting finding, we pursued the study of the polarographic reduction of other organic compounds. Of the functional groups found to be electroactive [Zuman (1969)] under the dropping mercury electrode, the formyl



group was chosen because of its important role in essential oils and other natural products [Fuson (1962)] and in the one-carbon transfer process (transformylation) in biochemistry [Mahler and Cordes (1971)].

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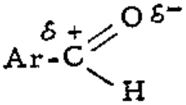
* For previous paper in this series, *see* Lim *et al* (1971). Philip. Jour. Sci. 100: 261-265.

The formyl group possesses a partially positive carbon



due to the inductive effect of the sp^2-oxygen. This makes carbon susceptible to reduction. Fourteen aromatic aldehydes with appropriate ring substituents were chosen as models and each was subjected separately to polarographic reduction under identical conditions.

EXPERIMENTAL PART

The following substituted benzaldehydes were all obtained from E. Merck, Darmstadt, Germany: 4-bromobenzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 2-nitrobenzaldehyde, 3-nitrobenzaldehyde, and 4-nitrobenzaldehyde.

Benzaldehyde was procured from Riedel-de Haen, AG, Seelze, Hannover, Germany while the three isomers of fluorobenzaldehyde were obtained from Sigma Chemicals Co., St. Louis, Missouri, USA. The other aldehydes, 2-bromobenzaldehyde, and 4-hydroxybenzaldehyde were procured from Pfaltz and Bauer, Inc., Flushing, New York.

All these aromatic aldehydes were found to be polarographically pure as shown by the absence of interfering waves during the reduction process. The identity and purity of these compounds were confirmed by infrared spectroscopy and melting point determination.

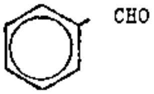
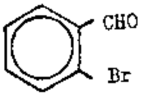
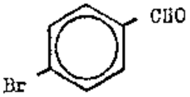
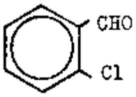
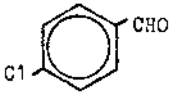
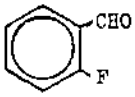
The instruments used in this study are: E.H. Sargent Model XXI Polarograph, Perkin-Elmer Infracord Spectrophotometer, Thomas Hoover Capillary Melting Point Apparatus, and Beckman "Zeromatic SS-3" pH meter.

The substrates were separately prepared at a concentration of 10^{-3}M in 20-per cent alcohol buffer (phosphate), pH 7.00, containing 0.01-per cent gelatin. Each sample was deaerated with purified nitrogen gas for 4 minutes before polarographic reduction in an H-cell containing a saturated calomel electrode. All measurements were made at 24 to 25°C at a drop time of 2.1 seconds, a voltage range of zero to 1.70 volts and a sensitivity of 0.200 μa/mm. The half-wave potentials were calculated following the standard procedure [Manual for Sargent Model XXI Polarograph (1952)].

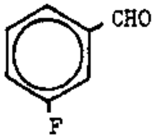
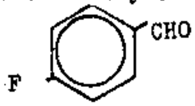
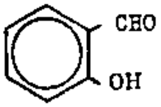
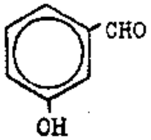
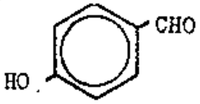
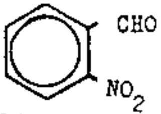
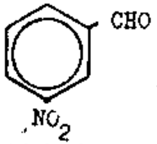
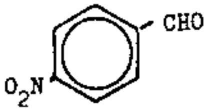
RESULTS AND DISCUSSIONS

The half-wave potentials, $E_{\frac{1}{2}}$, of the different aromatic aldehydes are shown in Table 1.

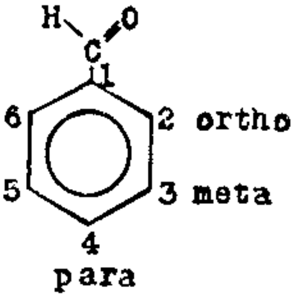
TABLE 1.—*Half-wave potentials (vs. S.C.E.) of benzaldehyde and some of its derivatives (10^{-3} M solution in 20-per cent alcohol containing 0.10 M KH_2PO_4, 0.042 M NaOH, and 0.10-per cent gelatin).*

Compound	$-E_{\frac{1}{2}}$ (volt) for the formyl group[1]	Standard Deviation of the Mean (volt)
 Benzaldehyde	1.323	0.0015
 2-bromobenzaldehyde, or o-bromobenzaldehyde	1.129	0.0013
 4-bromobenzaldehyde, or p-bromobenzaldehyde	1.234	0.0021
 2-chlorobenzaldehyde, or o-chlorobenzaldehyde	1.147	0.0017
 4-chlorobenzaldehyde, or p-chlorobenzaldehyde	1.254	0.0013
 2-fluorobenzaldehyde, or o-fluorobenzaldehyde	1.229	0.0021

(Table 1 Continued)

 3-fluorobenzaldehyde, or m-fluorobenzaldehyde	1.231	0.0019
 4-fluorobenzaldehyde, or p-fluorobenzaldehyde	1.355	0.0007
 2-hydroxybenzaldehyde, or o-hydroxybenzaldehyde	1.356	0.0012
 3-hydroxybenzaldehyde, or m-hydroxybenzaldehyde	1.298	0.0012
 4-hydroxybenzaldehyde, or p-hydroxybenzaldehyde	1.452	0.0010
 2-nitrobenzaldehyde, or o-nitrobenzaldehyde	1.335	0.0025
 3-nitrobenzaldehyde, or m-nitrobenzaldehyde	1.294	0.0013
 4-nitrobenzaldehyde, or p-nitrobenzaldehyde	1.348	0.0020

In the series of halogenated benzaldehydes, the decreasing ease of reduction (towards more negative $E_{\frac{1}{2}}$ values) is as follows:



o-F > m-F > -H > p-F

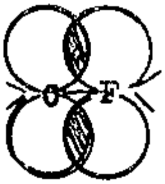
o-Cl > p-Cl > -H

o-Br > p-Br > -H

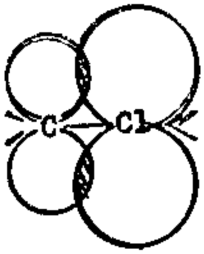
o-Br > o-Cl > o-F > -H

p-Br > p-Cl > -H > p-F

The net electrical effect of halogen atoms when bound to a pi-system always provides an enigmatic experience [Ferguson (1963)]. The halogens are generally electron-releasing through resonance in the order: F > Cl > Br > I. However, these are also electron-withdrawing by inductive effect. The extent of electron donation depends on the extent of maximum overlap between the p-orbital which contains the lone pair of the halogen and the p-orbital of the carbon to which the halogen is attached. The fluorine atom exhibits more π-electron delocalization than chlorine, bromine or iodine because its p-orbital is of the same size as the p-orbital of the adjacent carbon atom. The result is maximum overlap. As the halogen size increases, the extent of overlap decreases and consequently, its tendency to donate electrons also decreases.



C-F



C-Cl

The electron-attracting effect of the halogens depends on the electronegativity of the atom and the distance of this atom from the reactive site. Since resonance and inductive effect oppose each other in the halobenzaldehydes, one can deduce the net effect of the substituents from the $E_{\frac{1}{2}}$ values. From this we can assess the relative action of each halogen at the reactive site.

The order of the ease of reduction follows more an inductive order than steric order. The more positive the carbonyl carbon of the formyl group becomes, the more susceptible it is to reduction. This inductive order is enhanced by steric effects exerted by the substituent at the *ortho* position. The bulkier the *ortho* substituent, the greater is the decrease in its resonance contribution. Perhaps, this is due to steric inhibition of resonance brought about by the "buttressing effect" [Sylianco (1971)]. The disalignment of p-orbitals caused by the "buttressing effect" would decrease π-electron delocalization.

The "*ortho*-shift" [Ku and Lim (1971)] values in Table 2, show that all *ortho* substituents in this study enhance reactivity regardless of the nature of the substituent. This is not similar to the effect of *ortho* substituents on the reduction of nitroarenes [Ku and Lim (1971)] where the halogens gave negative *ortho*-shifts. The only difference between the nitro group and the formyl group is that the former is reduced at a much lower (less negative) potential.

With benzaldehyde as the reference, one can observe the following: the *para*-fluoro substituent is more electron-donating than hydrogen whereas all the other halogen substituents regardless of being *ortho*, *meta*, or *para* are more electron-attracting than hydrogen.

For the *ortho* substituents, the observed effect is a composite of the opposing resonance and inductive effects plus steric effects. This steric inhibition of resonance is no longer operating in the *meta* and *para* substituted models. All the *meta* subs-

TABLE 2.—*Shifts in half-wave potentials.*

Substituent	*Ortho*-Shift*	Δ_o**	Δ_p**	Δ_m**	Δ_o/Δ_p
F	0.126	—0.094	0.032	—0.092	—2.94
Cl	0.107	—0.176	—0.069	——	2.55
Br	0.105	—0.194	—0.089	——	2.18
OH	0.096	0.033	0.129	—0.025	0.26
NH_2***	0.013	0.012	0.025	—0.029	0.48

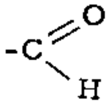
* Obtained from $(E_{\frac{1}{2}})_{o\text{-}X} - (E_{\frac{1}{2}})_{p\text{-}X}$, [see Ku and Lim (1971).]

** Obtained from $(E_{\frac{1}{2}})H - (E_{\frac{1}{2}})X$

*** Reduction of the nitro group

tituents are electron-withdrawing because direct resonance effect is not operating. Only inductive effect due to the electronegativity of the atom bound to the ring is present. When the *para*-substituents are considered, fluorine becomes electron-donating. Here, steric effect is absent and the predominating effect is resonance and not inductive effect. The fluorine is already three carbon atoms away from the formyl group so that inductive effect would be smaller than that of the *ortho*-isomer. Chlorine and bromine exert electron-attraction because resonance would be of lesser significance since these are atoms larger than fluorine.

The amino group is considered instead of the nitro group because from experience, the polarographic reduction of pure nitrobenzene gives only two inflections with $E_{\frac{1}{2}}$ values below -0.800 volt [Kolthoff and Lingane (1962), Perrin (1965), Zuman (1967)]. The end product of the reduction was reported as aniline [Kolthoff and Lingane (1962), Perrin (1965), Zuman (1967)]. Reduction of benzaldehyde [Kolthoff and Lingane (1962)] was reported to give only one inflection with an $E_{\frac{1}{2}}$ value at about -1.30 volts which is higher than those observed in nitrobenzene under comparable conditions. This was also noted recently when the formyl group was used as a substituent in nitroarenes [Ku and Lim (1971)]. These findings are taken to mean that the substituent affecting the reduction of

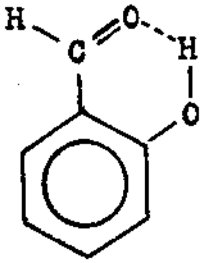


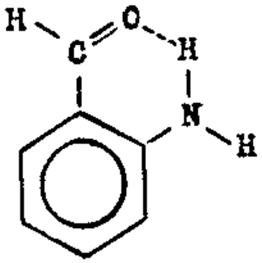
in nitrobenzaldehydes is no longer $-NO_2$ but $-NH_2$ [Zuman (1967)]. Besides, it was also separately observed by Gergely and Iredale (1953) that the $E_{\frac{1}{2}}$'s of the formyl group in the electroreduction of nitrobenzaldehydes are equal to the $E_{\frac{1}{2}}$'s of the corresponding aminobenzaldehydes.

The observed trend in the ease of reduction of the hydroxy and nitrobenzaldehydes are:

$$\text{m-OH} > \text{-H} > \text{o-OH} > \text{p-OH}$$
$$\text{m-NH}_2 > \text{-H} > \text{o-NH}_2 > \text{p-NH}_2$$

Hydroxy and amino groups are electron-donating groups when bound to a pi-system. This trend indicates that *meta*-hydroxy and *meta*-amino substituents are electron-attracting since at the *meta* position there is no direct resonance effect which donates electrons to the ring. The *ortho*-hydroxy and *ortho*-amino are more electron-withdrawing than their *para* counterparts because of intramolecular hydrogen bonding which increases the inductive effect on the formyl group. Such





intramolecular H-bonding cannot exist in the *para*-isomers.

It is also observed that the amino group is more electron-attracting than the hydroxy group in the six benzaldehyde derivatives studied (Table 2). The fact that oxygen is more electronegative than nitrogen should produce the reverse trend. Perhaps, this could be due to the greater acidity of the -OH group than the $-NH_2$ group. The medium becomes more basic as the reduction proceeds and there is more probability for -OH to exist as $-O^-$ than for $-NH_2$ to exist as $-NH^-$. In this case the oxygen function becomes more electron-donating than the amino function.

A linear free energy correlation following the method of Hammett was performed. From Figs. 9-10, we can observe that σn values [Wells (1963)]. give a better correlation than σ values [Wells (1963)]. The applicability of σn suggests the absence of direct conjugative interactions and indicates therefore that inductive effect rather than resonance is the predomi-

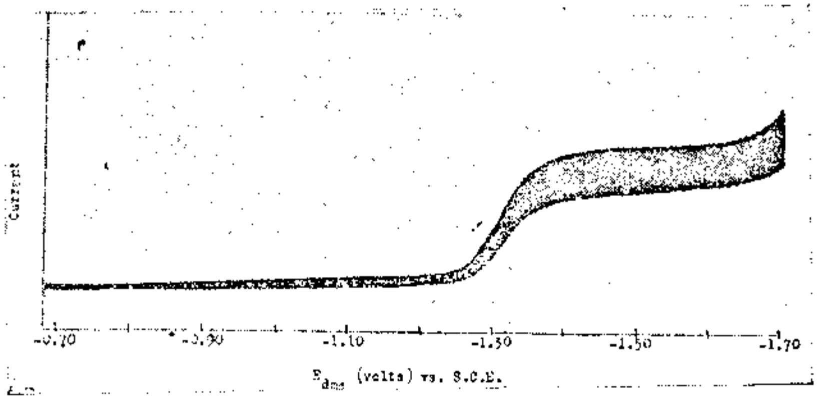


FIG. 1. Polarograms of 10^{-3} M benzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.042 M NaOH containing 0.01-per cent gelatin.

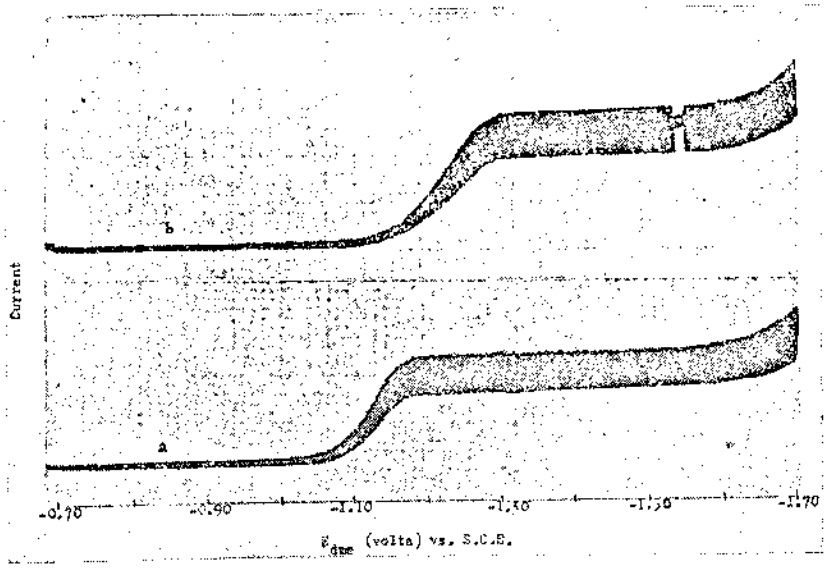


FIG. 2. Polarograms of 10^{-3} M (*a*) O-Bromobenzaldehyde, (*b*) p-Bromo benzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.042 M NaOH containing 0.01-per cent gelatin.

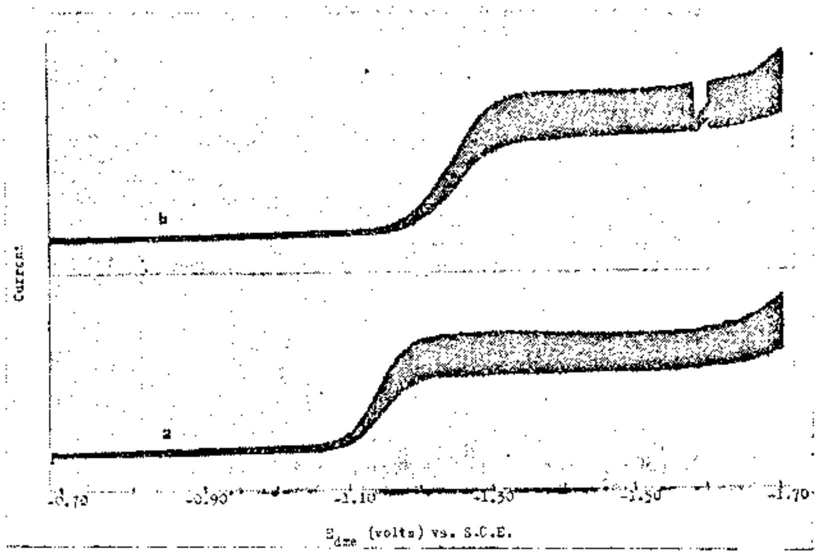


FIG. 3. Polarograms of 10^{-3} M (*a*) o-Chlorobenzaldehyde, (*b*) p-Chlorobenzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.042 M NaOH containing 0.01-per cent gelatin.

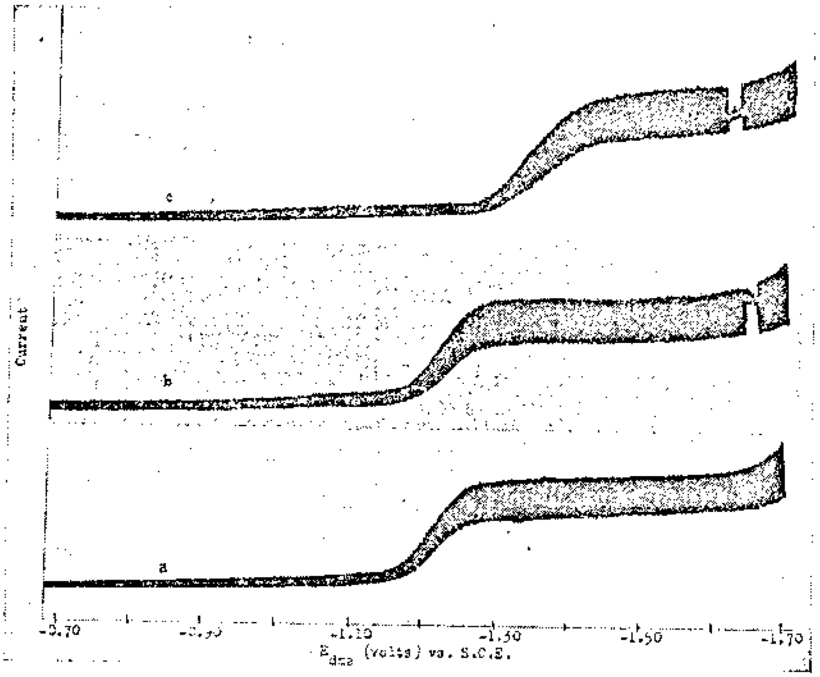


FIG. 4. Polarograms of 10^{-3}M (*a*) o-Fluorobenzaldehyde, (*b*) m-Fluorobenzaldehyde, (*c*) p-Fluorobenzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.042 M NaOH containing 0.01-per cent gelatin.

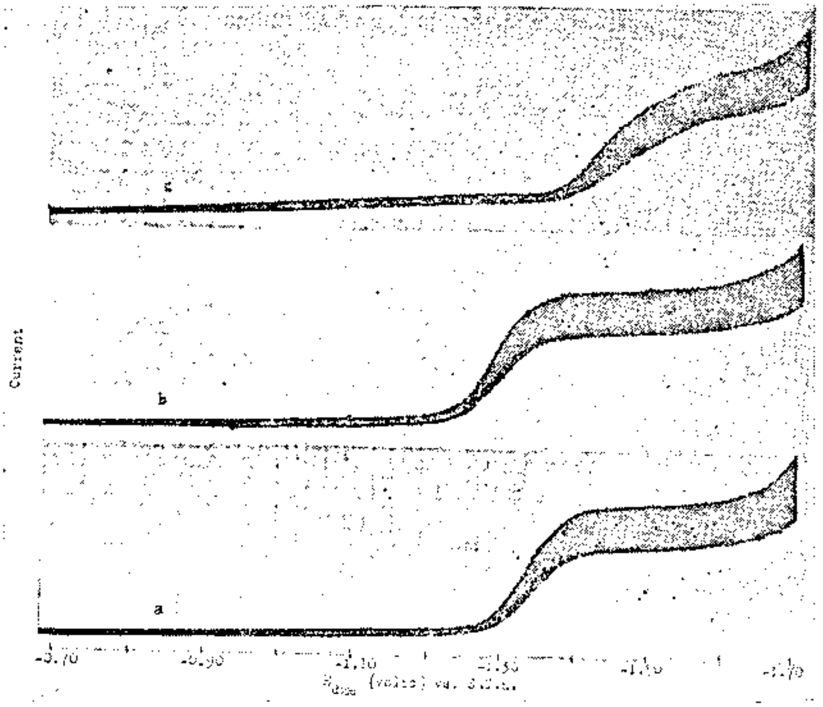


FIG. 5. Polarograms of 10^{-3} M (*a*) Salicylaldehyde, (*b*) m-Hydroxybenzaldehyde, (*c*) p-Hydroxybenzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.042 M NaOH containing 0.01-per cent gelatin.

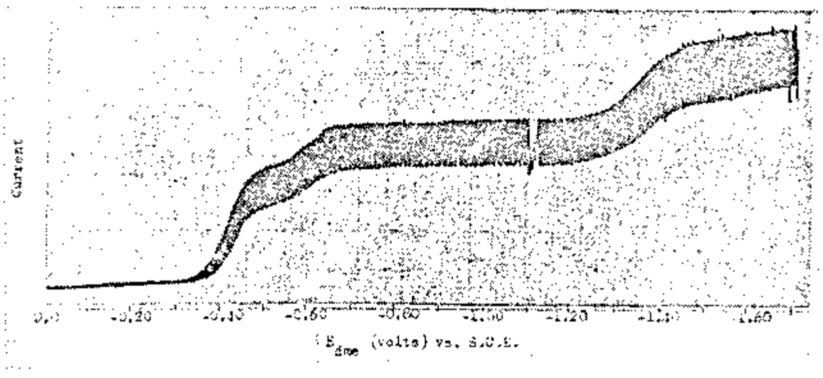


FIG. 6. Polarograms of 10^{-3} M o-Nitrobenzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.42 M NaOH containing 0.01-per cent gelatin.

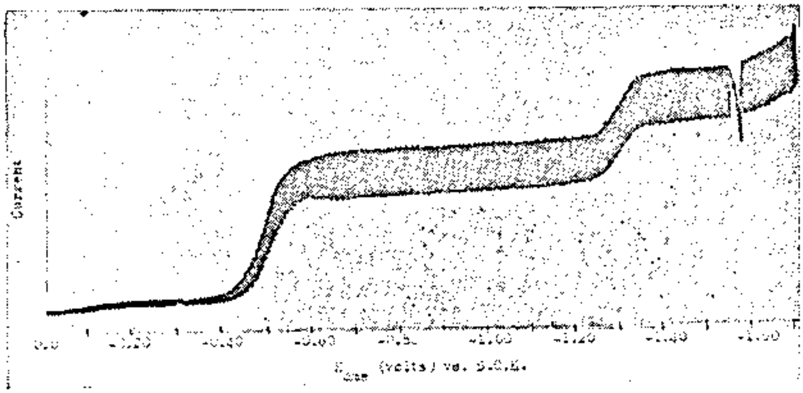


FIG. 7. Polarograms of 10^{-3} M m-Nitrobenzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.042 M NaOH containing 0.01-per cent gelatin.

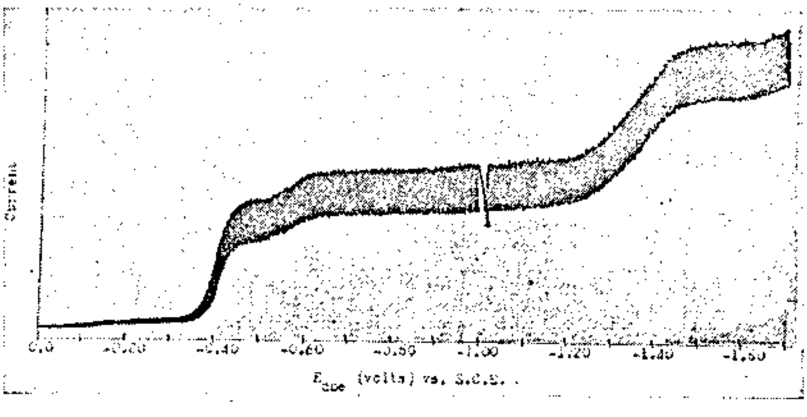


FIG. 8. Polarograms of 10^{-3} M p-Nitrobenzaldehyde in 20-per cent alcohol, 0.10 M KH_2PO_4-0.042 M NaOH containing 0.01-per cent gelatin.

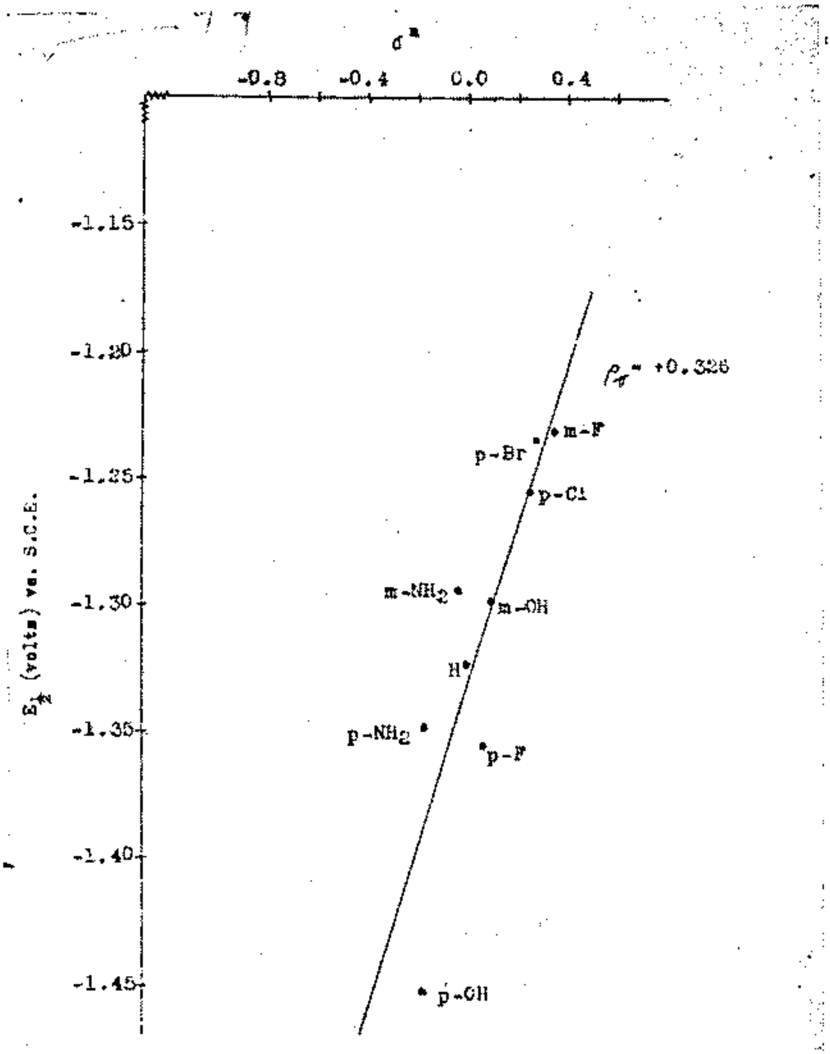


FIG. 9. Plot of half-wave potentials ($E_{\frac{1}{2}}$) vs. sigma-normal (σ n) values.

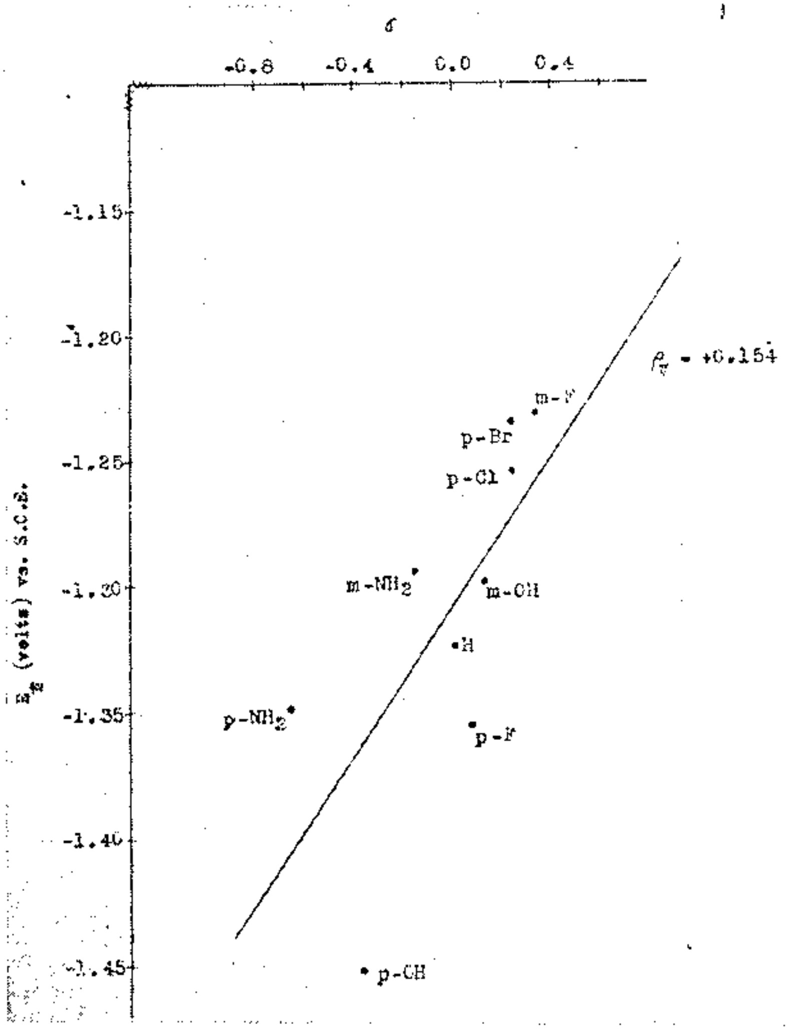


FIG. 10. Plot of half-wave potentials ($E_{\frac{1}{2}}$) vs. Hammett sigma (σ) values.

nating factor in the polarographic reduction of aromatic aldehydes. A calculation using the method of least squares was done. The correlation of $E_{\frac{1}{2}}$ and σ'' is given by the equation:

$$E_{\frac{1}{2}\,(X)} = -1.332 + 0.326\ \sigma''_{(X)}$$

The other values obtained are $P\pi$ = +0.326 volt, r = +1.028 volts and s = ±0.015 volt. Since the sigma values are not known with certainty, another line was used which has been found to be better suited for the calculation of new substituent constants [Jaffe (1953)]. The slope b of this line is $r''/_{P\pi}$ and the resulting equation is:

$$\sigma = 4.319 + 3.245\ E_{\frac{1}{2}}$$

Since no σ_o values for this particular benzenoid series or for a completely analogous system were available, the above equation was used to calculate σ_o constants for the benzaldehyde derivatives studied. The values are listed in Table 3. The method of multiple linear correlation was then used to check the correlation between σ_o, σ_I, and σ_R. A good linear correlation was obtained as indicated by a coefficient of linear multiple correlation of σ_o on σ_I and σ_R equal to -0.9665. The calculated σ_o values are therefore already independent of the van der Waals radius and other proximity effects, and are solely a function of electrical effects [Charton (1969)]. The σ_o values obtained give further indication that *ortho-* and *para-*electrical effects are different.

The observation that m-nitrobenzaldehyde (Fig. 7) gave only two waves as compared to o- and p-nitrobenzaldehyde, (Figs. 6 and 8) which gave three waves, suggests a new mechanism for this electroreduction:

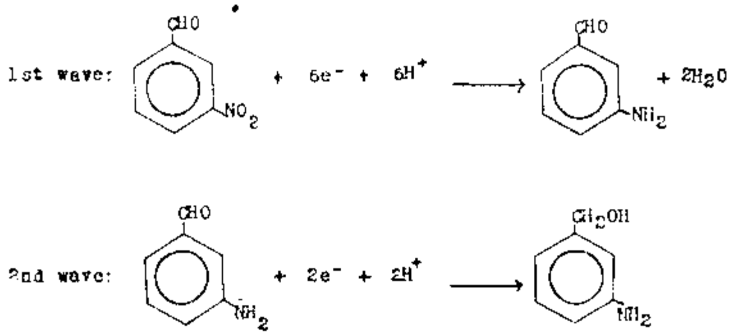


TABLE 3.—*Ortho-substituent constants.*

Substituent	σ_o
F	+0.332
Cl	+0.598
Br	+0.656
OH	−0.080
NH_2	−0.013

This study provided a lot of information on the behavior of aromatic aldehydes under polarographic reduction conditions. One may also predict that for the *meta*-halogen substituted benzaldehydes, the order of reactivity would be

m Br > m-Cl > m-F

if the trend in $E_{\frac{1}{2}}$ values of the halogens studied and the linear free energy correlation using substituent constants were to hold. Predictions for other derivatives can also be done on the basis of the LFER established for the system.

Ortho-effect has been observed with the *ortho*-halobenzaldehydes while intramolecular H-bonding has been directly inferred for the o-hydroxy and o-amino benzaldehydes.

The only limitation met in this study is that the effect of groups more electron-attracting than the formyl group cannot be studied as these are reduced before the formyl group is reduced. This was shown to be the case in the study of the nitro group.

ACKNOWLEDGMENT

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trol of wood-boring pests affecting tropical and subtropical forests.

Biologists, insect curators and students will not only find this work useful in the classification and identification of rhyssine specimens collected in the field or reared from their hosts; but it will facilitate recognition of new species.—Clare R. Baltazar

BOOK REVIEW

Books reviewed in this section represent a selection from those received from time to time by the National Institute of Science and Technology and the Philippine Atomic Energy Commission, two sister agencies under the National Science Development Board.

Ichneumologia Orientalis or a Monographic Study of Oriental Ichneumonidæ. Part II. The Tribe Rhyssini (Hymenoptera: Ichneumonidæ) By M. K. Kamath and V. K. Gupta. Published by the Association for the Study of Oriental Insects, c/o Dept. of Zoology, Univ. of Delhi, Delhi, India. Oriental Insects Monograph No. 2, 1972, 300 pp., 182 illus. Price $16 or £6.20, 100 Rs in India.

This monograph is a taxonomic revision of the Indo-Australian Rhyssini, a group of ichneumonid wasps parasitic on wood-boring sawflies and beetles. By referring to the maps, one can see at a glance the relative distribution of species and subspecies under each genus. Altogether there are 87 species in seven genera recorded in the Oriental and Australian regions. About half of this number are described by the authors as new. The adults are readily recognized by the sharp transverse wrinkles on the mesonotum, a feature seen only in tribe Rhyssini and in one genus (*Pseudorhyssa*) belonging in the tribe Theriniini. One begins to wonder that the significance of these sharp wrinkles is inasmuch as this characteristic remains unobserved in other parasitic wasps.

This work is similar, in presentation and thoroughness, to the monographs published by Dr. Henry Townes under whom the junior author trained. The tribe and genera are well characterized. Recognition of species becomes easy because there are keys to genera, species and subspecies and good illustrations are included. Each species is described together wtih variations observed among males and females. The study is comprehensive enough and it seems unnecessary to refer to past literature mentioned in the monograph.

Host association is entirely unknown for the species studied except for *Rhyssa persuasoria himalayensis*, therefore, this would be an open field of study for those interested in biological con-

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CHECKLIST OF THE ANTS (HYMENOPTERA: FORMICIDAE) OF ASIA. By J. W. Chapman and S. R. Capco. Institute of Science and Technology Monograph 1 (1951) new series. Paper, 372 pages. Price, $2.00, United States currency.

NOTES ON PHILIPPINE MOSQUITOES. XVI. GENUS TRIPTEROIDES. By F. E. Baisas and Adela Ubaldo-Pagayon. Institute of Science and Technology Monograph 2 (1952) new series. Paper, 198 pages with 23 plates and four text figures. Price $2.50, United States currency.

A REVISION OF THE INDO-MALAYAN FRESH-WATER FISH GENUS RASBORA. By Martin R. Brittan. Institute of Science and Technology Monograph 3 (1953) new series. Paper, 224 pages with three plates and 52 text figures. Price, $2.50, United States currency.

SECURING AQUATIC PRODUCTS IN SIATON MUNICIPALITY, NEGROS ORIENTAL PROVINCE, PHILIPPINES. By Donn V. Hart. Institute of Science and Technology Monograph 4 (1956) new series. Paper, 84 pages with 22 text figures and eight plates. Price, $1.25, United States currency.

AN ECOLOGICAL STUDY OF THE KOUPREY, NOVIBUS SAUVELI (URBAIN). By Charles H. Wharton. Institute of Science and Technology Monograph 5 (1957) new series. Paper, 111 pages with 11 plates and 16 text figures. Price $1.25, United States currency.

FERN FLORA OF THE PHILIPPINES. By Edwin B. Copeland. Institute of Science and Technology Monograph 6, Vols. 1-3 (1958-1960) new series. Vol. 1, 191 p., Paper, Price, $1.25; Vol. 2, 193-376 p., Paper, Price, $1.75; Vol. 3, 377-577 p., Paper, Price $1.75, United States currency.

THE PHILIPPINE PIMPLINI, POEMENIINI, RHYSSINI, AND XORIDINI. By Clare R. Baltazar. National Institute of Science and Technology Monograph 7 (1961) new series. Paper, 120 pages with four plates. Price, $1.50, United States currency.

PACIFIC PLANT AREAS. Edited by C.G.G.J. Van Steenis. National Institute of Science and Technology Monograph 8, Vol. 1 (1963) new series. Paper, 246 pages with 26 maps. Price. $3.00, United States currency.

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THE ARCHAEOLOGY OF CENTRAL PHILIPPINES. By Wilhelm G. Solheim, II. National Institute of Science and Technology Monograph 10 (1964) new series. Paper, 235 pages with 29 text figures and 50 plates. Price, $3.00, United States currency.

SHIFTING CULTIVATION AND PLOW AGRICULTURE IN TWO PAGAN GADDANG SETTLEMENTS. By Ben J. Wallace. National Institute of Science and Technology Monograph 11 (1970) new series. Paper, 117 pages with 14 text figures and five plates. Price, $1.50, United States currency.

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